ASSESSING THE ROLE OF SONOGRAPHIC CERVICAL LENGTH MEASUREMENT & VAGINAL CYTOLOGY IN PREDICTION OF THREATENED PRETERM LABOUR

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DECLARATION

I **Dr.S.GOWRI** solemnly declare that the dissertation titled **ASSESSING THE ROLE OF SONOGRAPHIC CERVICAL LENGTH MEASUREMENT & VAGINAL CYTOLOGY IN PREDICTION OF THREATENED PRETERM LABOUR** has been prepared by me. This is submitted to the Tamilnadu Dr. MGR Medical University, Chennai in partial fulfillment of the rules and regulations for MD Examination in Obstetrics and Gynaecology. This has not been previously submitted by me for the award of any degree or diploma from any university.

Place : Chennai
Date : . 12.2010

CERTIFICATE

This is to certify that the dissertation titled **ASSESSING THE ROLE OF SONOGRAPHIC CERVICAL LENGTH MEASUREMENT AND VAGINAL CYTOLOGY IN PREDICTION THREATENED PRETERM LABOUR** is the bonafide work done by Dr.S.GOWRI between May 2009 to October 2010 during her M.D.O.G; Course at INSTITUTE OF SOCIAL OBSTETRICS, GOVT. KASTURBA GANDHI HOSPITAL, MADRAS MEDICAL COLLEGE, Chennai.

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CONTENTS

LIST OF ABBREVIATIONS

EI	**Esinophillic Index**
KPI	**Karyopyknotic Index**
MI	**Maturation Index**
TVUS	**Transvaginal Utrasound**
CGA	**Cervical Gland Area**
LBW	**Low Birth Weight**
IUGR	**Intrauterine Growth Retardation**
IVH	**Intraventicular Hemorrhage**
RDS	**Respiratory Distress Syndrome**
URINE C/S	**Urine Culture and Sensitivity**

INTRODUCTION

INTRODUCTION:

"GOOD PRENATAL SUPERVISION, EARLY DIAGNOSIS, AND TREATMENT GOES A LONG WAY IN AMELIORATING MANY CASES SO THAT FETO-MATERNAL OUTCOME IS SATISFACTORY"

-PRITCHARD, 1978

Any endeavor to reduce perinatal mortality calls for a successful effort to reduce problem of preterm birth, for no single obstetrical misfortune is more wasteful as preterm birth. Half of neonatal morbidity occurs in preterm infants.

Preterm birth occurs in about 12% of all pregnancies varying from 5-12% . Preterm labor is defined as occurrence of regular uterine contractions (4 or more in 20 minutes; 8 0r more in 60 minutes) and cervical changes (effacement> 80% and dilatation > 1cm) in females with intact membranes and gestational age <37wks and pregnancy > 28wks.

THREATENED PRETERM LABOR:

If uterine contractions are perceived in absence of cervical change the condition is called threatened preterm labor. This condition tends to be over diagnosed and over treated and an objective assessment may reduce unnecessary intervention. Early identification of at risk gravidas with timely referral for sub specialized obstetrical care may help to identify women at

risk for preterm labour and delivery and there by reducing morbidity, mortality and expense associated with prematurity.

NEED FOR STUDY:

In past, traditional approach to diagnosis of preterm labor in females with no apparent cervical changes was to observe them for variable period of time and repeat digital examination looking for effacement and dilatation.

This approach to the diagnosis of female with contractions and minimal or no cervical changes by digital examination has changed radically at present to determine who among them are destined to deliver prematurely, it is necessary to perform examination of cervix by TVUS and vaginal cytology adds value to the study.

Digital assessment of the cervix has been commonly used to diagnose premature labour or to evaluate women perceived to be at increased risk of preterm labour. Digital assessment of cervical length is subjective, varies between examiners, and underestimates true anatomic length. Digital exams before

Hysterectomy underestimated cervical length by approximately14mm, whereas transvaginal ultrasound measured length accurately. Investigations using transvaginal ultrasound measurement as the standard confirmed that digital examination underestimates cervical length. This underestimation may result from an inability to digitally assess the cervix length beyond the

vaginal fornices unless there is two cm or more of dilation and the entire intracervical canal are examined.

COMPARISON BETWEEN TRANSVAGINAL, TRANSABDOMINAL, AND TRANSPERINEAL ULTRASOUND CERVICAL MEASUREMENT

Ultrasound assessment of the cervix was initially transabdominal, but specific disadvantages led to a preference for the transvaginal examination.

Transabdominal ultrasound requires filling the bladder to assess the cervix adequately, but this may spuriously lengthen the cervix by opposing

the anterior and posterior lower uterine segments, concealing cervical shortening or funneling. In contrast, transvaginal ultrasound is performed with the bladder empty.

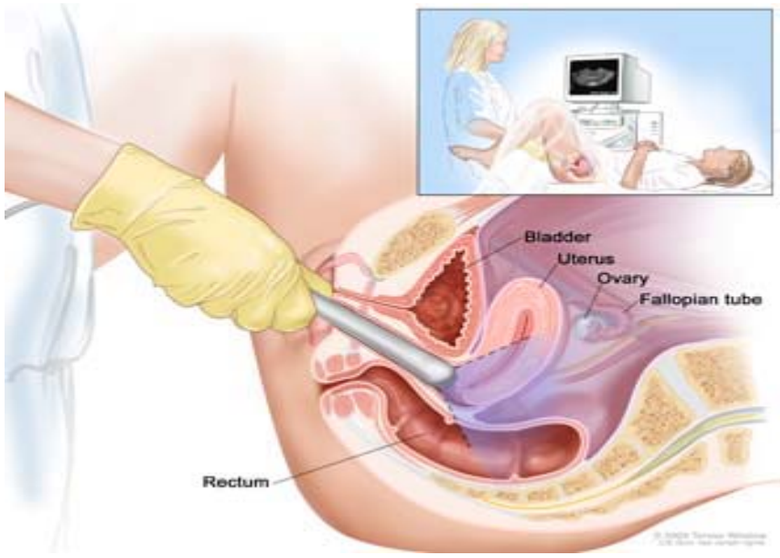
Transabdominal resolution is hampered significantly by maternal obesity, shadowing from fetal parts, and the need for lower frequency transducers.

Transperineal ultrasound has been performed as less invasive than the transvaginal examination but most studies have found that both approaches are acceptable to women. Since image resolution is better transvaginally, transperineal ultrasound should be reserved for and offered to women at increased risk of preterm birth for whom vaginal assessment is unacceptably invasive or uncomfortable.

NORMAL CERVICAL LENGTH

Cervical length is normally distributed and remains relatively constant until the third trimester. Heath found at 23 weeks a mean length of 38 mm. Iams found a mean length at 24 weeks of 35 mm and at 28 weeks of 34 mm. If funneling is present, measurement should exclude the funnel and be taken from the funnel tip to the external os.

TRANSVAGINAL CERVICAL MEASUREMENT WITH SUSPECTED PRETERM LABOUR



Transvaginal ultrasound is superior to digital assessment of cervical length but has limited ability to assess texture and dilation. Of all variables assessed by digital or ultrasound examination, transvaginal cervical length measurement is the best preterm birth predictor. Prediction of preterm birth by internal os funneling has been found by some investigators but not others. Cervical length greater than three cm has a high negative predictive value for delivery less than 34 weeks. This information may help patients avoid unnecessary interventions of unproven value such as tocolysis, hospitalization, and activity restriction. Randomized trials are needed to determine optimum Management following discovery of premature cervical Shortening by transvaginal ultrasound.

Cervical insufficiency defined as cervical changes in absence of uterine contraction. It was traditionally considered as mechanical/Anatomic defect that is either congenital/Acquired.

Current thinking favors a concept of functional insufficiency including a spectrum of progressive degrees of insufficiency rather than incompetence. Biochemical process involves inflammatory response with up regulation of cytokines/prostaglandins and matrix metalloproteinase resulting in premature cervix ripening. Process that initiate cervix change can be either loss of mechanical / functional support /infection. These painless cervix changes sooner or later result in premature myometrial contraction. It is recommended that 3measurements are taken 5minutes apart and lowest of 3 are considered.

PROCEDURE: - (VAGINAL CYTOLOGY BY PAP SMEAR)

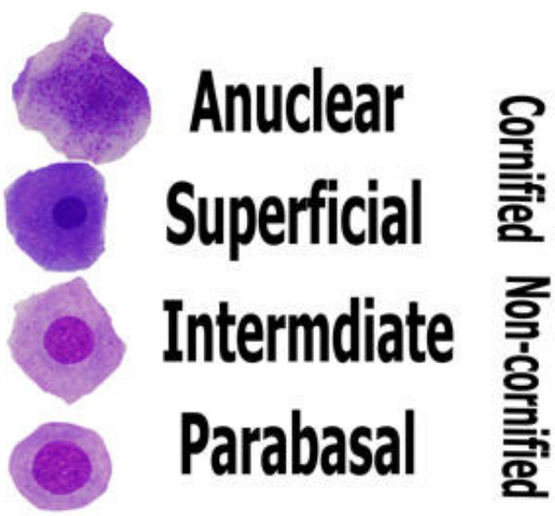
Contractions of sufficient frequency and intensity to effect progressive effacement and dilatation of the cervix at 28-37 weeks gestation are indicative of active preterm labour. If the diagnosis of preterm labor is suspected, but not confirmed, it many be prudent to fist obtain a vaginal smear of cytology before pelvic cervical examination. If the diagnosis of preterm labor becomes obvious after the pelvic examination, the vaginal smear specimen can be subsequently discarded. However, if the diagnosis

remains in doubt, the vaginal smear specimen can be sent to the lab for analysis.

CYTOLOGICAL EVALUATION OF FETAL MATURITY BY STUDYING THE EFFECTS OF HORMONES IN LATERAL VAGINAL WALL SMEAR

Smears are taken from the upper two thirds of the lateral vaginal wall by a wooden or plastic spatula. Immediately a smear is made from this on a clean glass slide. The slide is immersed in 95% ethanol (without drying it). Normal vaginal epithelium is lined by a stratified multilayered squamous epithelium. The thickness and structure of the epithelium is closely related to the conc. of the circulating ovarian hormones.

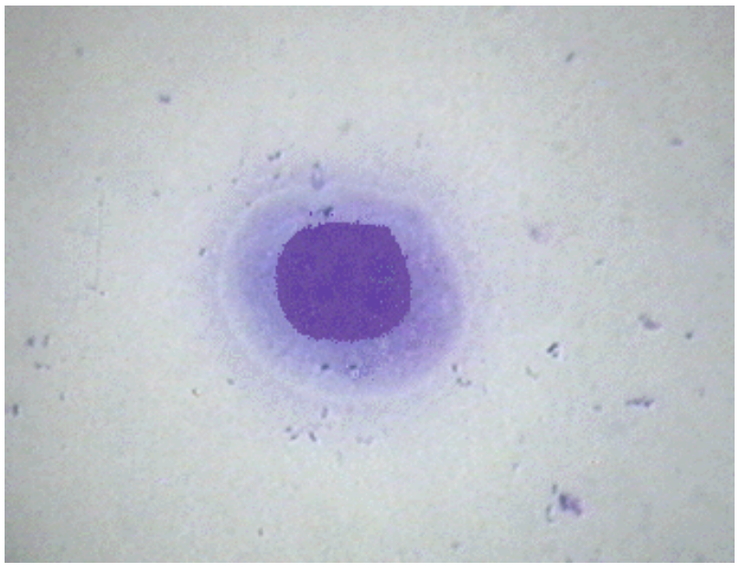
Under the influence of unopposed estrogen (no opposition by progesterone e.g., in the proliferative phase) due to the absence of progesterone, this vaginal epithelium is fully mature and fully developed and has 4 layers e.g.,



1) THE BASAL CELL LAYERS (THE RESERVE CELL LAYER): This layer consists of ONE ROW of cells. These are very small cuboidal cells with relatively large nuclei. They are firmly attached to basement membrane and not exfoliated. THIS CELL LAYER DOES NOT EXFOLIATE. The regeneration of vaginal epithelium occurs from the basal cell layer.

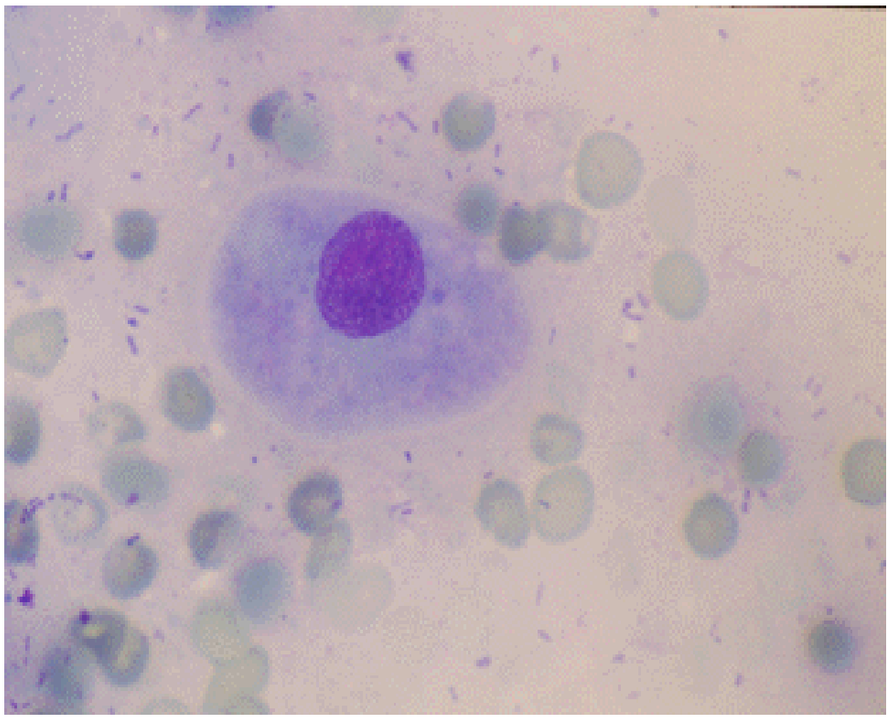
2) THE PARA BASAL CELL LAYER: This layer is composed of several ROWS of cells. They are slightly larger with central nuclei also slightly larger. Para basal cells exfoliate. The exfoliated Parabasal cells are squamous, small, round or oval. They stain blue against a clear and clean background of the vaginal smear. Cytoplasm is compact, central nuclei relatively large. Presence of exfoliated Parabasal cells in large numbers in vaginal smears means ovarian inactivity (no hormone stimulus e.g., immediately after delivery.)

PARABASAL CELL



3)THE INTERMEDIATE CELL LAYER: This layer is composed of several rows of cells. These are flatter in shape. Nuclei are vesicular. Cells are rich in glycogen content under the influence of larger amounts of progesterone and low amounts of estrogen, this layer is especially well developed and thickened. The presence of large number of intermediate cells against a dirty and messy background of Doderlein bacilli/cell debris/mucus /leucocytes—means progesterone influence as in the 2nd half of ovulatory menstrual cycle (i.e. post ovulatory menstrual cycle).

INTERMEDIATE CELL



There is a special and particular form of intermediate cell i.e. curled cell form of intermediate sq cell called Navicular cell. Navicular cell is oval (boat) shaped cell originally described by Papanicolaou in 1925. The cell border of this cell is thickened and prominent. Cytoplasm stains light. Nuclei are vesicular and eccentric. If there is subsequent insufficient progesterone production without simultaneous estrogen reduction-the above smear will have-

1-disappearance of navicular clusters

2-breaking up intermediate cell clusters

3-replacement of intermediate cells by superficial squamous cells

Exfoliated intermediate squamous cells are slightly smaller cells. Stain pale

blue. Cytoplasm is less transparent and rather denser. Nuclei are vesicular. These cells have a tendency to curl at the edges. These cells are “sticky” in nature and so they adhere to each other and clump together in masses thus forming clusters. They exfoliate also in clusters. Navicular cells when found in vaginal smear are found in clusters. These navicular cells are found-

1-in hormonally normal pregnancy in large numbers

2-in excessive androgen production due to progesterone effect

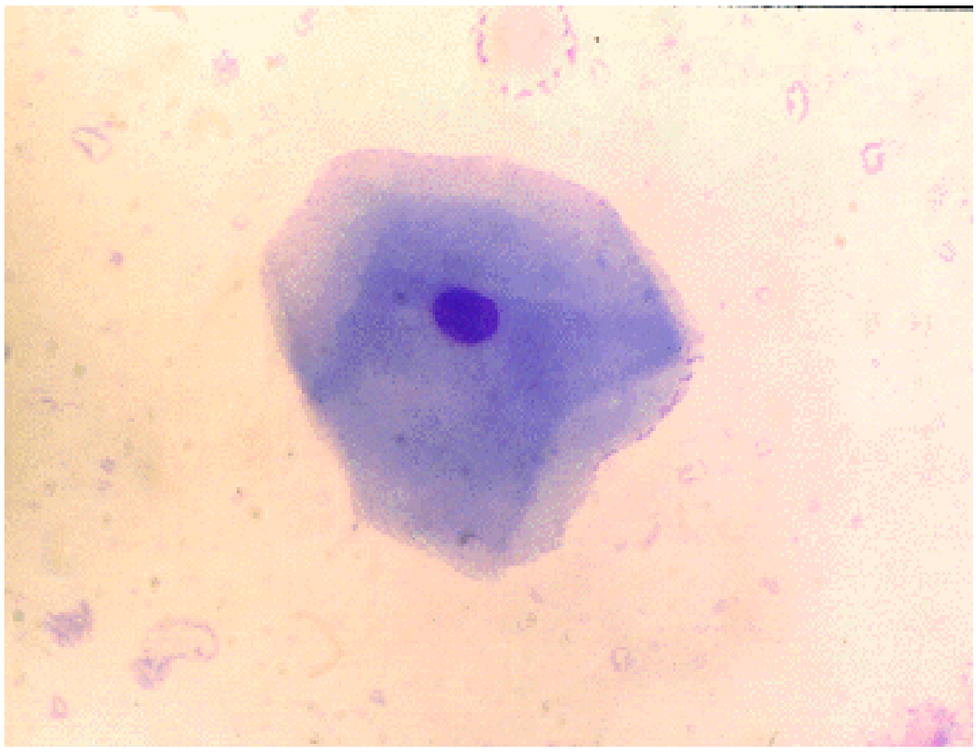
3-O+P pill

4-low estrogen status(with progesterone)

Navicular clusters are always found whenever there is a marked thickening of the intermediate squamous cell layer of the vaginal epithelium.

4) SUPERFICIAL SQUAMOUS CELL LAYER: This layer is composed of few rows of cells. These are large flat cells with pyknotic nuclei. The percentage of squamous epithelial cells having this nuclear pyknosis is known as karyopyknotic index (KI). The percentage of superficial squamous cells showing cytoplasmic acidophilia is known as eosinophilic index (EI) or cornification index (CI). Sometimes this layer contains anucleated keratinized cells in the topmost layer. Estrogen is the only hormone that promotes vaginal squamous proliferation to the superficial layer (means progesterone is either low or absent simultaneously).

SUPERFICIAL CELL



Exfoliated superficial squamous cells are seen as large polyhedral-flat-lying singly. Nuclei are pyknotic, cytoplasm thin, transparent stains pink with Pap staining technique. These are most mature cells of the vaginal epithelium. There presence in the smear means highest degree of vaginal proliferation-unopposed estrogen influence (no or lack of progesterone to oppose estrogen) simultaneously e.g., proliferative / 1st half of normal menstrual cycle / estrogen administration / estrogen producing tumor / some androgen due to estrogen effect and other conditions associated with excessive estrogen production.

There is no characteristic pattern if there is lack of estrogen while

progesterone levels are maintained or if the quantities of both hormones are simultaneously reduced.

PARABASAL CELLS ↓ A shift towards this A shift to left

INTERMEDIATE CELLS ↓ A shift towards this Shift to midzone

SUPERFICIAL CELLS ↓ A shift towards this Is shift to right

A shift towards to right If from right then a midzone shift to left -then it is midzone shift to the right If from left then a midzone shift to the right

In hormonally normal pregnancy. Due to large amounts of progesterone (corpus luteum in 1st trimester and placenta later on). And large amounts of estrogen---- placenta and the fetus.

Karyopyknotic cell index (K.P.I): - It was estimated by the ratio of total karyopyknotic cells(KC) to the total vesicular(intermediate) cells(VC) in a sample.

K.P.I=KC/VC

Vaginal smears are seen as:

<u>1st Trimester (Corpus Luteum Phase):</u>

1) progressive enlargement of intermediate cell layers because of progesterone influence of post ovulatory phase.

2) more dense clustering of these intermediate cells

3) marked decrease in superficial cells (present from preovulatory phase of

menstrual cycle)

4) E.I. falls below 5% and K.I. not > 10%

Mid pregnancy smear (from 3rd month onwards until 2-3 months prior to term):

1) large numbers of clusters of intermediate cells are formed

2) large numbers of thick clusters of navicular cells are formed. Navicular cells appear after 3rd week

3) no superficial cells are formed (because large amounts of estrogens are opposed by large amounts of progesterone)

4) E.I and K.I are very low --- each < 10%

5) background is dirty and messy as said earlier

At Term Smear (from 38-40 wks—beginning fall of progesterone by placenta):

1) less no of navicular cells

2) small no of individual clusters of navicular cells

3) discrete intermediate squamous cells

4) maturity index slightly shifts to right

5) E.I rises to > 5-6% (even 10 %)

6) K.I rises to about 15-20 % means impending delivery

Beyond Term Smear:

1) no intermediate cells

2) no navicular cells

Post Term Smear:

1) no intermediate cells

2) no navicular cells

3) no superficial cells

4) considerable number of parabolas cells appear so there is significant shift of M.I to “left” : 100/0/0-means fetus in danger and deliver the patient immediately.

5) Nearly all cells are single

But these are not always accurate and reliable tests so not used routinely. **MATURATION INDEX (M.I.):** It is the expression of percentage of the 3 major types of cells exfoliated from the stratified squamous epithelium of vaginal walls eg. Parabasal/ Intermediate/ Superficial cells

The order is just like above.

Each cell type has been given a Maturation value

Parabasal =0 intermediate =0.5 superficial=5.0

In mid cycle of normal ovulatory menstrual cycle the M.I =0 / 40 / 60

In 1st half of menstrual cycle M.I=0 / 0 / 100----completely right

At midcycle (i.e. at ovulation) it is 0 / 40 / 60 ---slight shift to right ---so a shift of M.I to midzone

In 2nd half =0 / 70 / 30 ------shift to left in midzone

In hormonally normal pregnancy =0 / 95 / 5----shift to midzone

Soon after conception---progesterone is secreted by the corpus luteum. So M.I shifts towards the midzone 0 / 70 / 30 it does not go in reverse order i.e. to preovular pattern.

It continues to be there throughout pregnancy 0 / 95 / 5 due to massive levels of O and P and other cortical steroids. It continues it's midzone shift until usually within a few weeks of delivery i.e. it reaches it's characteristic pattern of pregnancy i.e. an extreme shift to midzone of M.I to 0 / 95 / 5. This pattern is not reversed towards the preovulatory pattern as in normal menstrual cycle.

TRANS VAGINAL ULTRA SOUND:-

POSITION: Female in recumbent position, bladder empty.

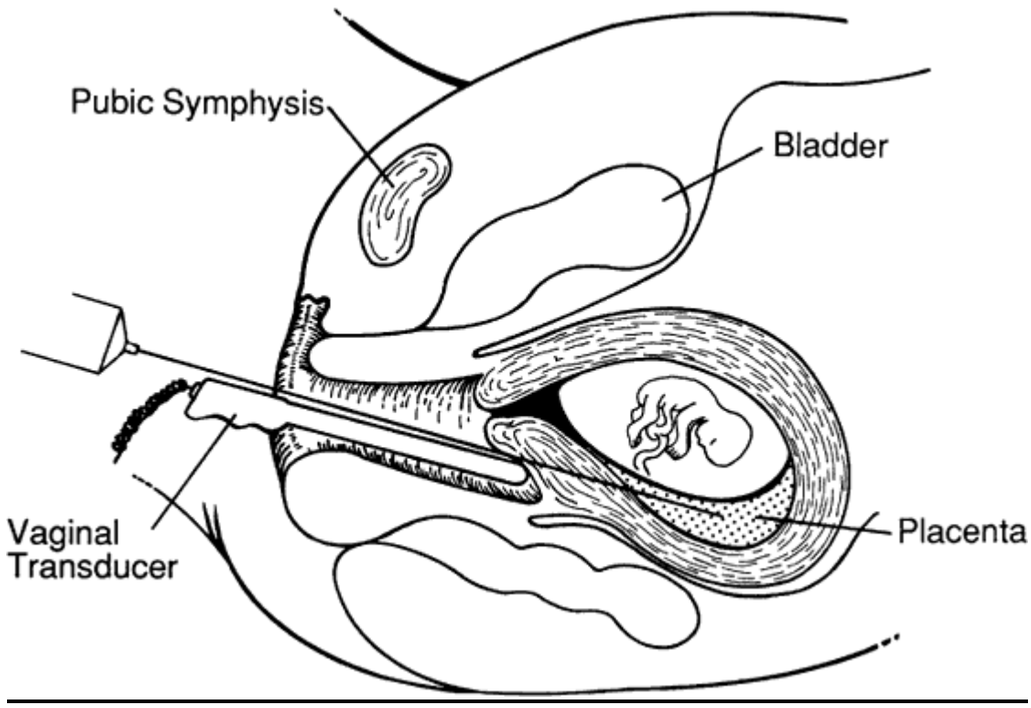
EQUIPMENT: 5MHZ/7MHZ Vaginal probe transducer.

STEPS: Probe inserted into vagina and advanced until cervix is visualized in sagital plane with echogenic endocervix mucosa along endocervix canal. Then probe is moved back in vagina and reapplied against cervix using minimal/no pressure. Length of endo cervix canal, width of internal OS and length of funnel if present are noted. Most important is measurement of cervical length:-Measured from internal OS to notch made by external Os.

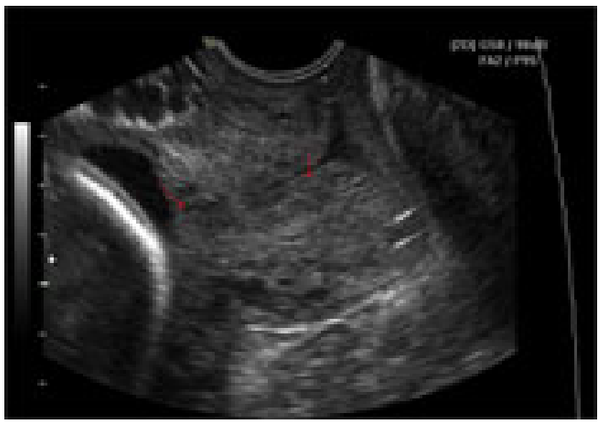
Funneling if present: - Cervix length is between upper and lower end of closed segment of endocervix canal.

Width of Internal OS: - Measured from anterior to posterior lip of cervix.

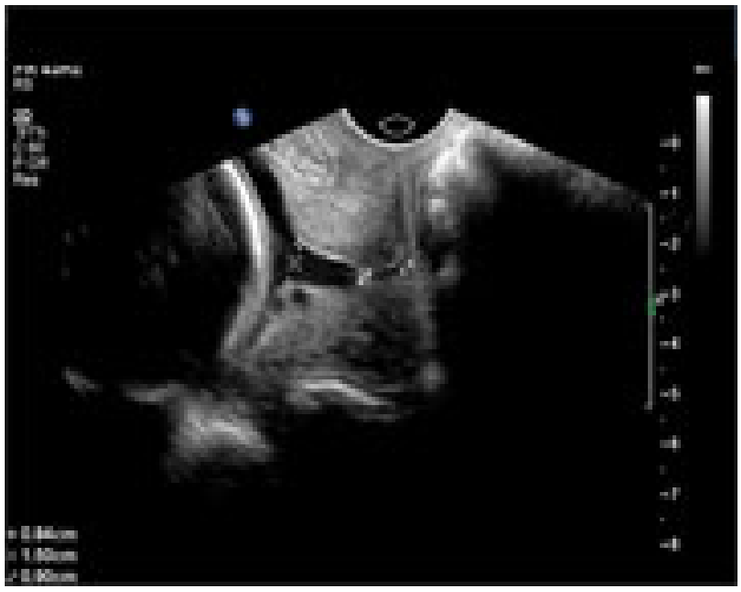
TRANS VAGINALULTRA SOUND IN PREGNANCY



Transvaginal cervical length measurement from the internal to external cervical os (arrows).



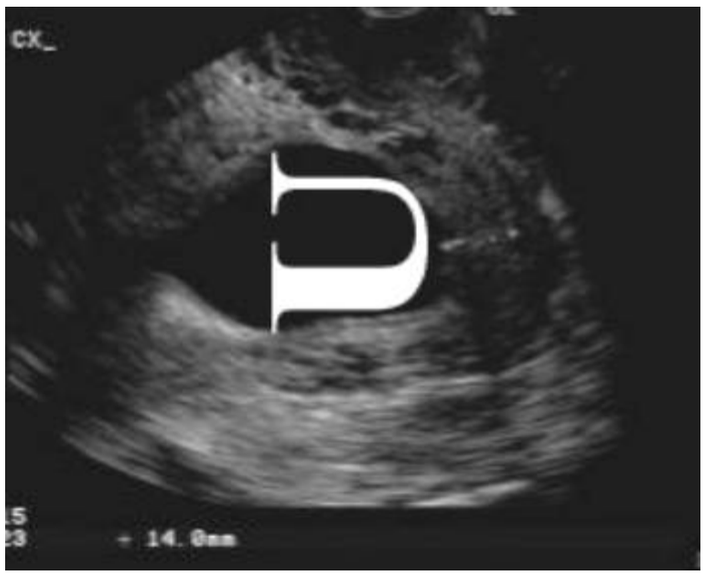
There is a cervical funnel with a length of 1.50 cm and a width of 0.84 cm. The remaining cervix measures 0.90 cm.



A cervical funnel extends to the external cervical os.

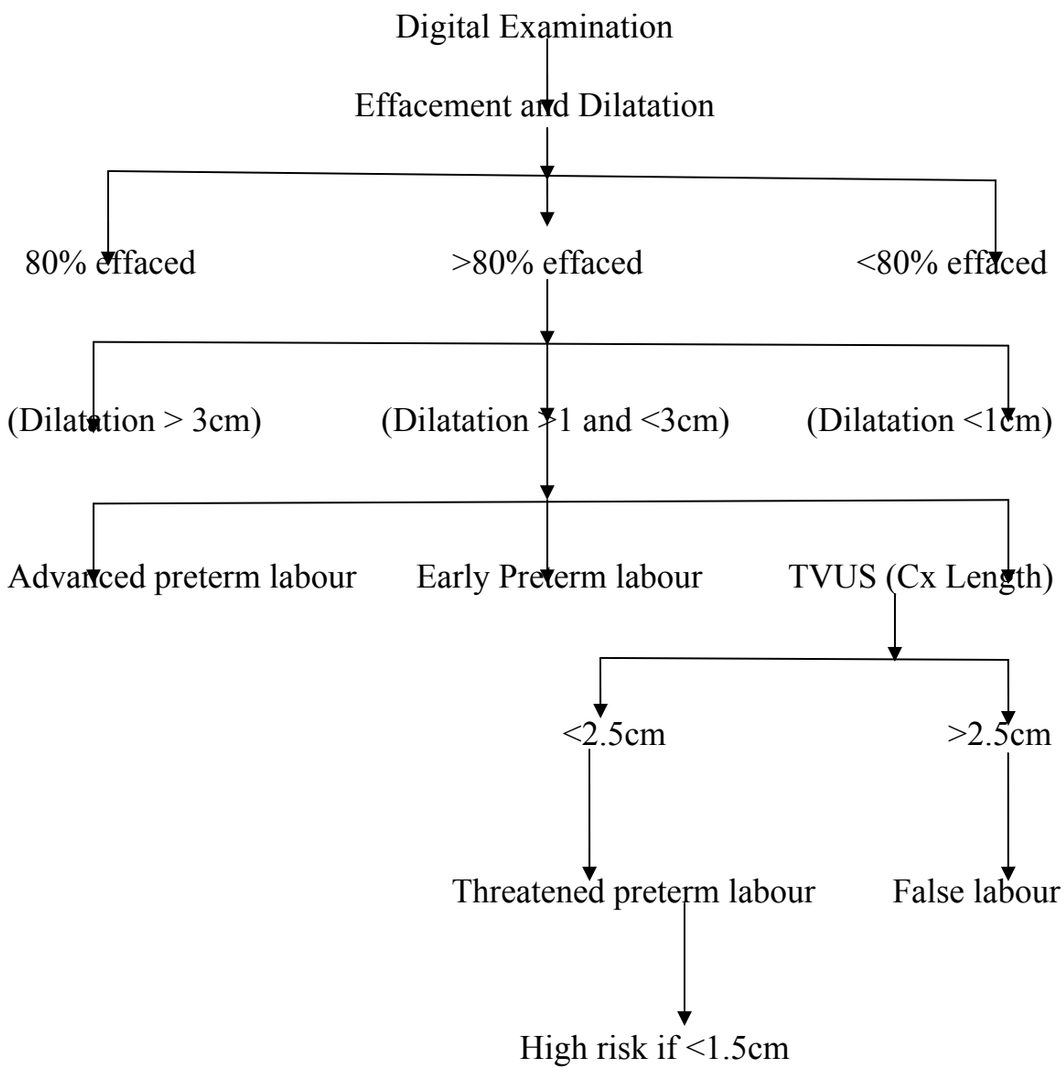
<u>Minimal endocervical canal dilatation that has been associated with an increased risk of preterm labor.</u>

THE SHORT CERVIX:



Funnel length:-Will be distance between imaginary line used to measure width of internal OS and upper end of closed segment and endocervical canal. Examination takes not more than 5 minutes and causes minimal or no discomfort to patient. The cervical length slowly decreases from mean of 4cm at 16wks to 3cm at 40 wks and there is no significant difference by parity. Since risk of preterm birth increases markedly when cervix < 2.5 cm, this measurement is widely accepted threshold to define risk of premature birth. Possibility of preterm delivery(Positive Predictive Value) when cervical length is less than 25mm is 17.8% , Negative Predictive Value of cervical length >25mm is 97%. So these females are reassured and do routine care.

DIAGNOSIS OF PRETERM LABOUR



AIM &OBJECTIVES

AIM &OBJECTIVES:

All patients with features of threatened preterm labour are studied. In this study we investigate potential value of cervical length by Trans Vaginal Sonography & determination of vaginal cytology in prediction of outcome of threatened preterm labour.

To facilitate early recognition of the condition and there by providing prophylactic pharmacologic therapy to prolong gestation and reduce the incidence of RDS and improve neonatal outcome.

REVIEW OF LITERATURE

REVIEW OF LITERATURE:

Iams et al (1990) used vaginal ultrasonography to measure the length of the cervix and also documented the incidence of spontaneous delivery before 35 weeks gestation. They examined 2915 women at approximately 24 weeks of gestation. They examined 2915 women at approximately 24 weeks of gestation and 2531 of these women again at approximately 28 weeks. Spontaneous preterm delivery (at less than 35 weeks) occurred in 126 of the women (4.3 percent) examined at 24 weeks. The length of the cervix was normally distributed at 24 and 28 weeks [mean+/- SD], 35.2 +/- 8.3 mm and 33.7 +/- mm, respectively). The relative risk of preterm delivery increased as the length of the cervix decreased. When women with shorter cervixes at 24 weeks were compared with the women with values above the 75th percentile, the relative risks of preterm delivery among the women with shorter cervixes were as follows: 1.98 for cervical lengths at or below the 75th percentile (40mm), 1. 35 for length at or below the 50th percentile (35mm), 3.79 for lengths at or below the 25th percentile (30mm), 6.19 for lengths at or below the 10th percentile (26mm), 9.49 for lengths at or below the 5th percentile (22mm), and 13.99 for lengths at or below the 1st percentile (13mm) ($p<0.001$ for values at or below the 50th percentile). The risk of spontaneous preterm delivery is increased in

women who are found to have a short cervix by vaginal ultrasonography during pregnancy. Shows the distribution of subjects among the different percentiles and relative risk for spontaneous preterm birth.

An inverse relationship between cervical length measurement and relative risk of preterm birth was demonstrated. Using receiver operator curves, three potential thresholds values for clinical use were identified. 30mm, 25mm and 20mm.

VARIABLE	**≤ 20 mm**	**<25 mm**	**< 30 mm**
Sensitivity	23%	37.2%	54%
Specificity	97%	92.2%	76%
Positive predictive value	25.7%	17.8%	93%
Negative predictive value	96.5%	97%	97.4%

The rate of spontaneous delivery before 35 weeks was 4.3% among female examined at 24wks. The tenth percentile cervical length measurement at 24 weeks was found to be 25mm and this increased the risk for preterm delivery six fold. Although a cervical length measurement of 25mm had only an 18% positive predictive value, this measurement has

subsequently been used as a benchmark of short cervical length in second trimester in many studies.

Why is the Cervix short?

The length of the cervical canal measured by USG in the second and early third trimester ranges from 10 to 50mm, the median length is 35mm, the length percentile is 25mm and the 90th percentile is 45mm. The risk of spontaneous preterm birth increases as the length of the cervix decreases across the entire range of cervical length. A cervix length of <25mm at 22-24 weeks is associated with a six fold increase in preterm birth before 35 weeks relative to women whose cervical length is above the 75th percentile.

Some of the range of cervical length is thought to be simply biologic. In other cases, women may experience early effacement as a result of inflammation due to hemorrhage, infections , or less commonly due to biophysical effects of uterine distention or subclinical contractions.

1. Physical Factors:

A. Biological variation

1) Absolute versus relative insufficiency

2) Cervical function insufficient to carry a singleton pregnancy to 32 weeks but not to term.

a) Cervical function insufficient to carry a singleton pregnancy to 32 weeks but not to term.

b) Cervical function insufficient to carry a singleton pregnancy to 24 weeks, that is the lower most end of a bell curve of cervical performance.

B. Uterine Volume

a) Multiple gestations, especially higher order

C. Cervical Injury

a) Obstetric laceration

b) Gynecological conditions: conisation, laser or LEEP

D. Contractions

2. Biochemical Factors:

a) Infection

b) Decidual Hemorrhage

c) Role of genetic polymorphisms

In a low-risk population endovaginal cervical ultrasonography helps to rule out a preterm delivery if cervical length is long enough. It can also detect cervical incompetence. In a high-risk population, women whose cervix is longer than 30mm can be identified. These women have over 80% chance to deliver on or after 36 weeks of pregnancy.

Heath VC et al (1998): stated that cervical length at 23 weeks is <or = 15min in <2% of the populations; this group contains about 90% and 60% of the women delivering at <or = 28 and <or 32 weeks, respectively.

Measurement of cervical length provides accurate prediction of risk for early preterm delivery.

Transvaginal ultrasound measurement of the cervix is increasingly used for the prediction of preterm labour. In comparison to clinical vaginal examination, it has the advantages of being highly reproducible, with a low inter-observer variability, and of offering an evaluation of the entire cervical canal, including the internal os. The sensitivity and specificity of transvaginal ultrasound have been validated by several studies in women with symptoms of preterm labour, however its clinical applications and its limits have yet to be fully determined.

Hertzberg BS et al (1995) assessed the spontaneously changing gravid cervix its clinical implications and prognostic features. Sonograms in 27 pregnant patient with a spontaneously changing cervix were studied prospectively. The length and width of cervical funneling and the length of intact cervix caudal to the funneling were measured when the cervical dimensions were most normal and most abnormal.

Sonographic measurements were correlated with clinical and delivery data. Twenty patients delivered preterm, although only six delivered within a week of the ultrasound examination. Wider funneling of the internal os and a shorter segment of intact cervix caudal to the funneling both correlated with an increased likelihood of preterm delivery.

Most patients with a spontaneously changing cervix deliver preterm. Measurements obtained when the cervix appears most abnormal are most predictive of early delivery.

Is a study done by *Watson WJ et al* (1999): There was a positive association between a short cervix and increased risk of preterm birth (F=13.3, P<.0001). The variable with the highest predictive value for preterm birth was the cervical length at 24 weeks gestation. Changes over time did not substantially improve the predictive accuracy for spontaneous preterm birth.

We conclude that a short cervix as determined by endovaginal sonography has a significant association with preterm birth in a high-risk obstetric population. Measurements taken at 24 weeks gestation are most accurate in assessing this risk, and serial observations of the cervix over time have less accuracy for predicting preterm birth.

Taipale P et al (1998): stated that despite much research, little progress has been made in timely identification of the mothers at risk. He examined the uterine cervix with ultrasonography to discover whether such a procedure would be helpful in determining which women will deliver prematurely.

He performed transvaginal ultrasound examinations in addition to routine transabdominal ultrasonography at 18 to 22 weeks gestation in 3694

consecutive pregnant women with live singleton fetuses. He measured the length of the uterine cervix and evaluated the dilatation, if any, of the internal os. The results of cervical ultrasonography were not available to the clinicians. Spontaneous delivery occurred before 37 completed weeks in 88 women (2.4%) and before 35 weeks in 31 (0.8%). The relative risk of delivery risk of delivery before 35 weeks was 8 (95% confidence interval 1,267).

Transvaginal ultrasonography performed as an addition to routine transabdominal ultrasonography at 18 to 22 weeks helps to identify many patients at significant risk for prematurity; however, low sensitivity and low positive predictive value limit its usefulness in screening low-risk obstetric populations.

Fukami T et al (2003): Numerous reports have examined the relationship between sonographically determine cervical length and spontaneous preterm birth. Moreover, large screening studies have consistently demonstrated that the shorter the cervical length, the higher the rate of spontaneous preterm delivery. However, the sensitivity and positive predictive value of the cervical length for detecting preterm birth were low. Subsequently, developed a new sonographic cervical finding (shortened cervical length or absence of cervical glandular area) at 16-19 weeks gestation could predict spontaneous preterm birth. The absence of CGA as

compared to the shortened cervical length showed a higher sensitivity (75.0% vs. 50.0%) and a significantly elevated positive predictive value (54.5% vs. 8.3%) for preterm birth before 32 weeks gestation. It was concluded that the absence of CGA was a novel and useful sonographic parameter for predicting early spontaneous preterm birth.

In addition to primary predictors of preterm birth which are used to estimate the baseline risk of preterm birth, secondary predictors (based on examinations done during the current pregnancy) allow a more accurate assessment of the risk of preterm birth in individual women. Screening for early signs of spontaneous preterm labour has always been and important topic in obstetric care. During the last two decades, the detection of fetal fibronectin (FFN) from cervicovaginal detections and cervical shortening diagnosed by transvaginal ultrasonography have emerged as the major secondary factors of preterm birth.

Both markers have been extensively studied and consistently shown to be strong short term predictors of preterm birth across a wide range of gestational ages. Other secondary predictors that confirm the role of intrauterine infection in the pathogenesis of preterm birth are bacterial vaginosis (BV) and elevated levels of interleukin (IL-6, IL-8) ferritin and granulocyte colony-stimulating factor. Apart from bacterial vaginosis, inflammatory markers are still not routinely used.

The sensitivity of single markers in predicting preterm birth is only moderate and serial examinations of markers, combinations of different markers and multiple marker tests have been studied, with limited results. Studies of interventions in order to prevent preterm birth have also yielded mixed benefits, as a consequence of which the use of these markers to screen low risk pregnancies is generally not recommended.

Several investigators have attempted to use cervical length in asymptomatic women to predict preterm delivery.

Conoscenti et al (2003) prospectively followed 2469 women and found that cervical length at 13 to 15 weeks gestation was not different in women who delivered term and preterm.

Carvalho et al (2003) conducted a prospective study involving 529 pregnant women attending for routine antenatal care who underwent transvaginal scan at 11 to 14 weeks and 12-24 weeks for evaluation of cervical length. The mean cervical length was calculated at both steps of gestation and lengths were compared between groups which delivered at term and prematurely (<37weeks).

The mean cervical lengths were 42.4mm and 38.6mm at 11 to 14 weeks. Cervical length at 11 to 14 weeks was not significantly different between the groups who delivered at term (42+mm) and preterm (40.6mm). However, at 22-24 weeks evaluation, cervical length was significantly

shorter in the group which had a preterm delivery (26.7mm) and term (39.3mm). So he concluded that there is a spontaneous shortening of the pregnant cervix from the first to the second trimester of pregnancy.

Many studies have evaluated second trimester assessment of cervical length as a predictor of preterm delivery. Goldenberg et al conducted Preterm Prediction Study with the Maternal Fetal Medicine from 1993 to 1996.

They assessed about 3000 women for risk factors, biophysical characteristics and biochemical tests that might be predictive of preterm delivery. Using a cervical length of 25mm as the definition of short cervix (24 to 30 wks), positive fetal fibronectin was the strongest predictor of preterm birth followed by short cervix.

Among non-gravid women *Jackson et al* reported that transvaginal and transabdominal ultrasound measurements of the cervix agreed closely with anatomic measurements, whereas digital examination underestimated the cervical length by an average of 13.6mm.

Honest H et al (2003): Conducted studies where they undertook antenatal transvaginal sonographic cervical assessment among a population of pregnant women with known gestational age of delivery. There were 46 primary articles, which included a total of 31,577 women, consisting of 33 studies in asymptomatic and 13 studied in symptomatic women. Data were

extracted for the studies characteristics and quality. Accuracy data were used to form 2x2 contingency tables for various cervical length measurements with birth before 32, 34 and 37 weeks gestation as the reference standards.

Our review showed that transvaginal cervical sonography identifies women who are at higher risk of spontaneously preterm birth, although there was a wide variation amongst studies with respect to gestational age at testing, definition of threshold of abnormality and definition of reference standard.

The most commonly reported sub-group was testing of asymptomatic women at <20 weeks gestation using a threshold cervical length of 25mm with spontaneous preterm birth before 34 weeks gestation as the reference standard.

Both cervical length measurement and funneling, whether alone or in combination, appear to be useful (depending on the threshold chosen to define the abnormality) in predicting spontaneous preterm birth in asymptomatic women. For symptomatic women there was a paucity of data, although the degree of funneling appeared to be predictive of spontaneous preterm birth.

From *Goldenberg RC, Iams JD, Mercer BM et al*: The preterm prediction study: the value of new vs. standard risk factors in predicting early of all spontaneous preterm births (Am J Public Health 88:

233, 1998). In symptomatic women with suspected preterm labour a cervical length of <20mm is not necessarily predictive of preterm birth, but a length of > 30mm can reliable exclude preterm birth.

Hasegawa et al conducted a study in 729 pregnant women with no risk factors between 15 and 34 weeks gestation in Japan. Cervical parameters, including cervical length, internal os dilatation and funneling depth, more measured by transvaginal ultrasound. The predictive value of these measurements for preterm delivery was investigated in a prospective fashion.

Among various parameters, cervical length (mm) showed the best correlation with pregnancy outcome. The group with a shortened cervix (mean cervix length – ISD as cut off value) showed a significantly high preterm delivery rate exclusively in primigravidae (odds ratio: 4.86, 95%). Internal os dilation, in contrast, was a useful predictor in multiparous women (odds ratio 6, 98%). It was concluded that TVS cervical assessment, especially the measurement of cervical length, was effective for the prediction of preterm delivery in the primigravidae.

The accurate predictor, prophylaxis and management of preterm labour are a challenge for every obstetrician. This study conducted in a multi-disciplinary tertiary institute correlates cervical length by TVS with the occurrence of preterm labour.

Several published studies have demonstrated inverse relationship between cervix length and frequency of preterm delivery. The group with a shortened cervix showed a significantly higher preterm delivery rate exclusive in the primigravid population. In contrast, internal os dilation was a more useful predictor in multiparous women. The author concluded that the length of the cervix was possible an indirect indicator of cervical competence.

Although the predictive value of ultrasonography was low in this low risk population it is postulated that the predictive value will raise as the risk of prematurity in the study population increases.

Cervical effacement in pregnancy has been demonstrated by USG to begin at approximately 32 weeks for term births and as early as 16-24 weeks for preterm birth. The process of change of the internal os often is well established before recognition of external os changes. Cervical effacement may occur slowly and often precedes clinically evident preterm labour.

TRANSVAGINAL CERVICAL MEASUREMENT IN ASYMPTOMATIC PREGNANT WOMEN

Cervical length is inversely related to preterm birth risk in asymptomatic women. The largest study of this relationship noted relative risks, compared to women above the 75th percentile, of approximately four if length was less than 30 mm (25th percentile), six if less than 26 mm (10th

percentile), nine if less than 22 mm (5th percentile), and 14 if less than 13 mm (1st percentile). The positive predictive value was poor (35%). Heath and colleagues19 studied women who were not at increased risk of preterm birth and, using transvaginal ultrasound at 23 weeks, found that 1.7 percent had a cervix length less than or equal to 15mm. These women accounted for 90 percent of deliveries at less than 28 weeks and 60 percent of deliveries at less than or equal to 32 weeks. This suggests that the positive predictive value of a short cervix (≤15 mm) is much greater for extreme prematurity (≤28 weeks). The authors have created a formula to predict the risk of spontaneous delivery at less than or equal to 32 weeks based on cervical length at 23 weeks.

Although transvaginal ultrasound screening of cervical length can predict increased risk of preterm birth,23 there is no evidence that this information can be used to improve outcomes. Consultation and the proposed location of birth should be considered. Other management options, such as cerclage, activity restriction, tocolytics, and prophylactic steroids await appropriate evaluation by randomized trials. The significant association between cervical length and preterm birth risk may not apply to women who have undergone cervical surgery resulting in permanent shortening.

First detailed histologic description of changes in vaginal epithelium in pregnant female was made by *Faverger* in 1913.

Papanicolaou (1925) postulated that navicular cells might be used as diagnostic criterian for early pregnancy.

Pundel has emphasized the importance of obtaining vaginal scraping from lateral wall of vagina in its upper 1/3 rd and cites the loss of cell clustering and a sharp increase in KPI as among the most distinctive and significant changes related to impending onset of labor;

When smears are procured in this manner according to Pundel smears of late pregnancy contain <10% of superficial cells until shortly before onset of labor when there is rise of 15% or more.

Lemberg-Siegfried and *Samm* and *Pundel* suggest useful practical applicaton to be made from smears of late pregnancy, showing

1) Recognition of Impending onset of labour
2) Recognition of Post maturity of fetus
3) Progress fro success when medical induction of labour is contemplated *Barner* and *Zuspan* in applying the criteria of *Lemberg-Siegfried and Stamm*; were able to distinguish labour smears from prenatal smears with only 60% accuracy.

The typical smear in Normal Pregnancy shows a predominantly bluish green staining reaction and its cell population is almost exclusively derived from intermediate layer with large clusters of navicular cells. The cells have a pronounced tendency to crowd together in large clumps and curl their edges.

The pregnancy pattern described above denotes adequate progesterone effects. According to *Lichtfus*(1959) and *Pundel*(1959) the smear appearances change in the last 2 weeks before delivery.

The large clusters of navicular cells break up, into smaller clusters and later only isolated navicular cells are seen. Until they finally disappear altogether. At the same time there is generally less crowding pattern shows more discrete cells. Later still, the intermediate cells are replaced by superficial squamous so that eventually the smear pattern resembles that of the proliferative phase of the cycle. When this stage is reached, the onset of labour is expected within the next 48 to 72 hours.

Pundel described in 1959, the existence of the following three different smear types in the vaginal smear of a pregnancy near term and pointed out their practical significance.

PREGNANCY SMEAR BEFORE TERM:

This smear is the type which is found in the last trimester of pregnancy, namely exclusively intermediate cells, most of which are of the

navicular type and many exfoliated in placards. The acidophilic index is less than 2.3% the KPI under 10% multiple Doderlein bacteria are found in the background. This type of smear represents a hormonologically normal pregnancy with an adequately functioning placenta.

PREGNANCY SMEAR AT TERM:

A few days before normal labor starts spontaneously, the previously described smear will start to change. The cluster will be smaller. The karyopyknotic index will increase to 15-20%. The Doderlein bacilli will decrease in number and background will convey a “cleaner” impression. This type of smear indicates that the function of the placenta, particularly with respect to progesterone production, has decreased.

THE REGRESSIVE SMEAR:

In the regressive smear type not only have the placards disappeared and the intermediate cells been scattered discretely, but the number of acidophilic cells and pyknotic nuclei has increased considerably. The characteristic of this type of smear, however is the presence of basal and parabasal cells. In extreme cases red parabasal cells will be present-actually, the same cells that will be called ’lactation cells’ when found in a postpartum smear. This smear type may proceed to show the typical aspect of a postpartum smear and this seems to express the fact that as far as the vaginal epithelium is concerned, the pregnancy is hormonologically

terminated. The significance of this smear type is that it serves as a warning that placental function is completely exhausted.

Pundel (1959) found in 1000 patients who were clinically at term that 414 cases had the smear type of pregnancy before term, 574 that of pregnancy at term, and 12 the regressive type. Out of the 414 cases with the before term smear type only 18(4.3%) delivered spontaneously within 5 days after the smear was taken.

Of the 574 cases with the at term smear type, 528 (92%) delivered within 5 days. He also found that the spontaneously delivery of the 18 cases which were cytologically 'before term' could be attributed to nonhormonal factors such as hydramnios, toxemia and multiple pregnancy.

In the 46 with the at term smear type who did not start labour within 5 days 42 started labour between sixth and twelfth day after the first smear was taken. In two cases the smear showed a change towards the regressive type and labour had to be induced artificially.

From *Pundel's* material it appears that in over 90% of cases it can be predicted whether or not the patient will start spontaneous labour within 5 days. It should be understood that this is not entirely confirmed to the 40 weeks gestation. This change in smear type only reflects a decrease in placental hormonal activity and this may occur before the fortieth week.

In 12 cases with the regressive smear type, labour was artificially induced, but only eight babies could be saved. All twelve babies showed classical signs of post-maturity. Death of the four infants was attributed to post-maturity. This regressive smear type seems to be associated with serious fetal distress.

Pundel found in material of over 12,000 deliveries that true postmaturity existed in only 37 cases and in all cases the smear was of the regressive type. The smear reflects placental insufficiency and this is often accompanied by fetal distress or even death. But there are cases of fetal distress, for instance from erythroblastosis, malformations and infection which are not accompanied by placental dysfunction. In such cases the fetal distress may not show in the vaginal smear. After the baby dies from any cause however, the placenta will degenerated and evidence of this degeneration will be found in the smear.

` *Pundel*(1959) also obtained smears from 517 patients who had carried the pregnancy beyond the calculated day of delivery and who had been judged by skilled obstetricians to be ‘ripe’ for induction of labour 400 cases showed at term smear pattern 82 the pattern before term and 35 the regressive type of smear. In the82 patients who showed before term pattern the induction of labour was unsuccessful in any of the three attempts with pitocin. Of the 400 cases with the at term smear type 300 inductions were

successful at the first attempt and 98 on the second attempt, again without artificial rupture of membrane.

Prior to *Pundel's* publications in 1959, *Barnes and Zuspan*(1956) had published their results with this method. They reported an accuracy of more than 70% in determining the date of confinement, but doubted its use as a routine method. *Zidovsky*(1961) warned that traumatic lesions and infections may be responsible for the appearance of parabasal cells in the smears of women with prolonged pregnancy and this may make the interpretation less valid. *Nikilicek* (1963) however agreed with *Pundel* that these conditions can usually be recognized and seldom lead to errors of judgement. *Pundel's* cytologic conclusions depend on the assumption that prior to the onset of labour there is indeed a decrease of progesterone activity.

STUDY IN J.J. HOSPITALS BOMBAY

INTRODUCTION

The increasing levels of the hormones after conception bring about changes in the vaginal epithelium. They can be detected by examination of the vaginal smear which reveals intermediate cell-clusters and navicular cells. This cytological pattern changes suddenly at the end of pregnancy due

to drop in the. hormonal levels. The latter also brings about spontaneous labour within 5 days. However, there is no unanimity about the relationship between the changed smear pattern and the onset of labour.

In India, determination of biological pregnancy terns is of considerable practical importance, as many of the pregnant women attending general hospitals do not know the date of their last menstrual period. Also, in patients with irregular menses, the date of the last menstrual period cannot be relied upon to determine the expected date of delivery. In such case, to predict the date of labour, vaginal cytology is a simple and inexpensive parameter as compared to estimation of urinary total oestrogen, ultrasound studies and amniotic fluid analysis. In order to increase the predictability of vaginal cytology, in addition to subjective, classification of the smears into 'pre-term' and 'at-term' patterns, eosinophilic and karyopyknotic indices can be calculated

Lykke et al found that spontaneous preterm delivery, especially if the complications were severe. In a registry-based cohort study of 536,419 Danish women, delivery between 32 and 36 weeks of gestation increased the risk of preterm delivery in the second pregnancy from 2.7% to 14.7% (odds ratio [OR] 6.12; 95% confidence interval [CI], 5.84-6.42) and increased the risk of preterm birth from 1.1% to 1.8% (OR 1.60; 95% CI, 1.41-1.81). A first delivery before 28 weeks increased the risk of a second preterm

delivery to 26.0% (OR 13.1; 95% CI, 10.8-15.9) and increased the risk of preterm birth to 3.2% (OR 2.96; 95% CI, 1.80-4.88).

MATERIALS AND METHODS

Single smears were collected from 75 pregnant women in the age group of 18 to 40 years, attending antenatal clinic of J. *J. Group of Hospitals, Bombay*. The smears were obtained one week before the due date of delivery. They were collected from the upper one third of the lateral vaginal wall and were immediately fixed in ether-alcohol. The smears were stained with Papanicolaou method and were categorized into three patterns: 1.'Pre-term' type, when the smear showed large clumps of cyanophilic intermediate cells and navicular cells uniformly distributed on the slide; 2. 'At-term' pattern which revealed single intermediate cells or occasional small clumps of intermediate cells and a very few navicular cells; 3. 'Post-term' or 'regressive' pattern, in which the cellular characteristics were similar to those noted in the 'at term' category, but they were more clear and distinctive. Parabasal cells were also seen in these smears.

In addition to this categorization of the smears, eosinophilic and karyopyknotic indices were calculated. For this investigation, a total of one hundred cells were counted in four different microscopic fields under high power lens (magnification 40 x 10).

The time interval between the day of obtaining the smear and the

date of delivery was noted, and its relationship with the smear pattern was recorded. The data was analyzed by discriminant analysis.

RESULTS

Out of 75 smears examined, 37 were of 'pre-term' type, 34 showed 'at-term' pattern and 4 smears were of 'post-term' type.

The time interval between the date of obtaining the smear and the actual date of delivery is shown in.

For the statistical analysis, eosinophilic and karyopyknotic indices were calculated in each case. Discriminant analysis was carried out with this two indices. The separation function, T was obtained as:

$T = X + 0.8763Y$, where

$X = v$ Eosinophilic index

and

$Y = v$Karyopyknotic index

This produced a highly effective separation of 'pre-term' and 'at-term' smears. The discriminant function for 'pre-term' type: was 4.58 (Tp) and for 'at-term' pattern was 5.24 (Ta). The mean of the two discriminants was 4.91, which was the cut off value for 'pre-term and 'at-term' patterns. In our data, none of the 'at-term' patterns had discriminant: less than 4.91, whereas only one 'pre-term' pattern showed a value greater than 4.91 (i.e. 4.92).

DISCUSSION

The usefulness of vaginal cytology at the end of pregnancy for the prediction of labour has been studied by many workers. Some found it to be useful for the prediction of onset of labour whereas many others came to the conclusion that there was no relationship between the smear pattern and the onset of labour. Therefore, *Ortner* carried out discriminant analysis of eosinophilic and karyopyknotic indices and thus supplemented subjective criteria of cytological pattern determination with the objective criteria. The same method was followed by us. Our values of eosinophilic and karyopyknotic indices were lower than those obtained by *Ortner* and T value was also low. We could separate 'pre-term' and 'at-term' patterns quite well without any overlapping. Our prediction of onset of labour from the smear pattern was correct in 73% of the patients. Thus, we find that vaginal cytology at the end of pregnancy is a useful parameter to predict the onset of labour in most cases

Lichtfus recorded “at-term” changes in 97.3% and “prior-to-term” changes in 2.7% of patients within 5 days of spontaneous delivery in a series of 369 patients. In 88 patients examined by *Sammour* spontaneous delivery occurred within 5 days of the smear showing “at- term” changes in 73, or 83%, of patients.

In a study of 130 patients who had smears taken within 8 days, the spontaneous onset of labor, *Osmond-Clarke et al*, found that 8% showed a normal pregnancy smear of the clumped variety and 92% showed some cytologic changes-partly discrete smears in 34% and discrete smears in 58% of patients.

At variance with these reports are the findings of *Birtch* and *Abrams and Abrams*. Of 208 patients, *Birtch* obtained "before-term" smears in 53%, "approaching-term" smears in 42%, and "at-term" smears in only 5% of patients within 5 days of the onset of labour. *Abrams and Abrams* were unable to demonstrate the abrupt change from the third trimester to the "at-term" pattern within 5 days of the onset of labour in any of the smears from 193 patients.

Vaginal cytology changes during labor or immediately after delivery have been reported by *Birtch, Ruiz and Soule*. *Birtch,* in a study of 208 patients, recorded "before-term" smear in 50%, "approaching-term" smears in 29.3%, and "at-term" smears in only 20% of patients.

Ruiz studied smears from 200 patients and observed that during delivery vaginal cytology had the typical characteristics of pregnancy in 60% of patients, changes due to infection in 27%, and in only 13% of these patients described as precursors of imminent delivery.

Soule concluded that vaginal cytology obtained during spontaneous labor is of less correlative value than the clinical history.

Stricter criteria for a more reliable classification of smears are desirable because the partly discrete smears considered by *Osmond-Clarke et al*, as representing "prior-to-term" changes, might be interpreted as representing "approaching-term" or even "at-term" changes.

Nevertheless, there is no doubt that alterations in the vaginal cytology frequently occur towards the end of pregnancy. In 78% of patients reported here changes were found when the patients were in labor. If the patient were about to come into labor within 8 days, similar changes were found less often (62% of this series). It is likely that the changes would be detected more often before the onset of labor if smear could be taken every day or two rather than at weekly intervals, but this poses administrative problems that are likely to reduce the value of the method in general obstetric practice.

Neonatal Morbidity and Mortality by Gestational Age:

A study at University of California showing that between 24 and 33 weeks' gestation, benefits of tocolytic therapy are generally accepted to outweigh the risk of maternal and/or fetal complications and these agents should be initiated provided no contraindications exist. The following table

depicts survival, major short-term morbidity, and intact long-term survival by gestational age. The risk of neonatal mortality and morbidity is low after 34 completed weeks of gestation.

Gestational Age, wk	Survival	Respiratory Distress Syndrome	Intraventricular Hemorrhage	Sepsis	Necrotizing Enterocolitis	Intact
24	40%	70%	25%	25%	8%	5%
25	70%	90%	30%	29%	17%	50%
26	75%	93%	30%	30%	11%	60%
27	80%	84%	16%	36%	10%	70%
28	90%	65%	4%	25%	25%	80%
29	92%	53%	3%	25%	14%	85%
30	93%	55%	2%	11%	15%	90%
31	94%	37%	2%	14%	8%	93%
32	95%	28%	1%	3%	6%	95%
33	96%	34%	0%	5%	2%	96%
34	97%	14%	0%	4%	3%	97%

MATERIALS AND METHODS

MATERIALS AND METHODS:

This prospective clinical trial of 100 cases comprised of patients with feature suggestive of threatened preterm labour and multigravida with previous history of preterm labour who attended the Obstetrics and Gynaecology Department of Institute of Social Obstetrics and Kasturbha Gandhi Hospital for routine antenatal checkup.

The study was undertaken for a period of one year between May 2009-October 2010

Inclusion Criteria:

1) All Singleton live pregnancies with features of threatened preterm labour included regular uterine contractions, low abdominal cramping, low back pain, pelvic pressure & increased vaginal discharge.
2) Gestational age between 28weeks- 36 weeks
3) With Intact membranes
4) Cervical dilatation <3cms

Exclusion Criteria:

1) H/O 1st Trimester bleeding
2) Presence of uterine malformations and leiomyoma
3) H/O PIH, GDM, Essential HT
4) Multiple Pregnancies
5) Hydramnios

6) Low lying placenta
7) Pt in active labour, ruptured membranes.
8) Cervical Dilatation >3cms
9) Those who underwent permanent cervical shortening/surgery

Examination:

1) Maternal vital signs
2) Uterine activity, Tenderness, Fundal height, Presentation
3) Speculum examination to exclude rupture of membrane and assess cervical change
    a) High vaginal swab performed for culture and sensitivity
    b) Vaginal cytology if cervix <3cm dilated, no liquor/blood is visible

Investigation:

1) Midstream urine
2) High vaginal swab
3) Vaginal cytology
4) TVUS

VAGINAL CYTOLOGY:

For staining exfoliated cells in cytological specimens.

Summary and Explanation:

The Papanicolaou Staining procedure is used for examining exfoliated cells in secretions, exudates, transudates or scrapings of various internal organs and tissue. Cells are fixed to a slide and stained first with Hematoxylin, which stains the nuclei followed by **OG-6** and **EA-65** as a counter stain.

Reagents:

EA-65 Multiple Polychrome Stain

1000mL Catalogue # IMEA65

Light Green S.F. Yellowish, Fast Green FCF, Bismark Brown Eosin Y, Phosphotungstic Acid,Acid-Glacial Acetic Acid. **Filter before use.**

OG-6 Orange G Stain

1000mL Catalogue # IMOG6

Orange G, Phosphotungstic Acid. **Filter before use.**

Harris Hematoxylin

1000mL Catalogue # IMHARRISHX

Hematoxylin, Potassium Alum, Glacial Acetic Acid, Sodium Iodate. **Filter before use.**

EA-50 and **OG-6** were developed as general stains for vaginal smears. **EA-65**, a modification of **EA-50**, has been especially developed for use with OG-6 in the study of smears from urinary, paracentetic,

thoracentetic material, from sputum, from gastric and external ulcerated sources as well as those smears which, are heavily mixed with mucus. In such smears **EA-65** helps retain the translucency essential to proper cytological study. **EA-65** is interchangeable with **EA-50** in the staining procedure. Staining times and technique remain the same.

Storage and Stability:

Store at 15°-30°C. Reagents are stable until expiration dates shown on the label.

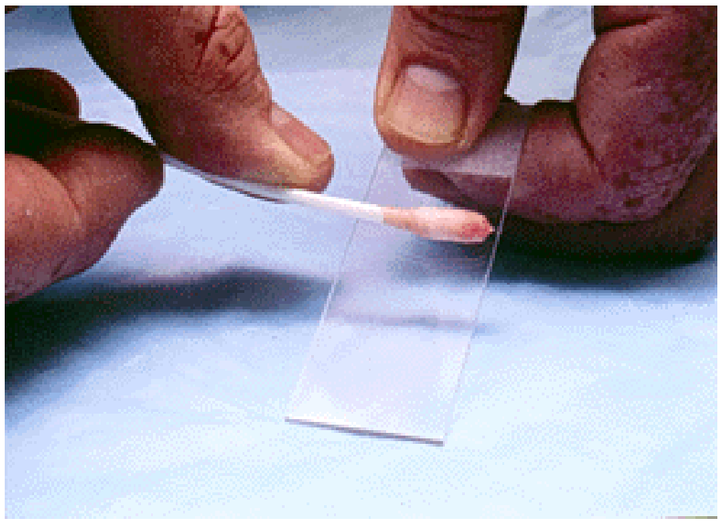
Warnings and Precautions:

Papanicolaou Staining reagents are flammable and toxic. Keep away from sources of ignition. In case of contact with eyes, rinse immediately with water and seek medical advice. May be fatal or cause blindness if swallowed. Dispose of waste in accordance with applicable laws.

Materials Required:

95% Ethanol, Hydrochloric acid, Cover slips, Microscope, Ammonium Hydroxide, and Microscope slides, Xylene Methanol.

Specimen Collection:

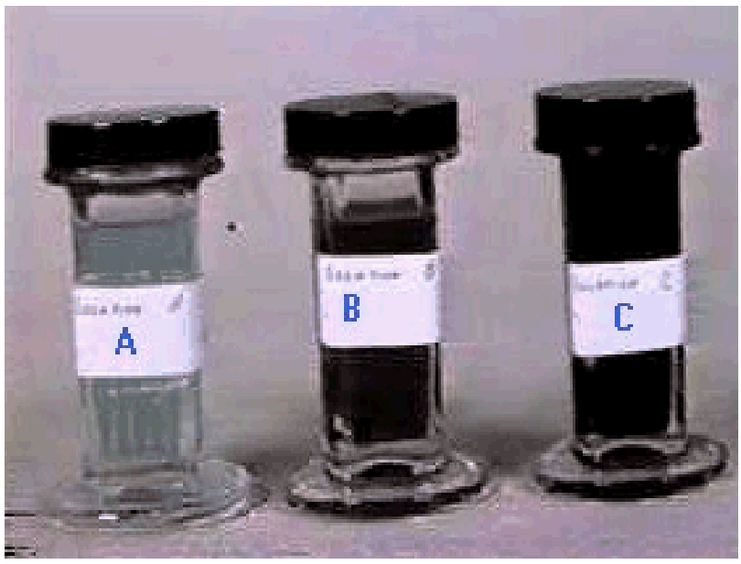


In the collection and preparation of smears for cytological examination, the major objectives are:

1. Specimens should have a sufficient number of cells from the upper third of lateral vaginal wall. Smears should contain well preserved cells uniformly distributed so that each cell can be individually examined.

3. The staining procedure should clearly define the details of all structures.

Cytological preparations are obtained from patient by approved methods and techniques. Scraping, obtained from the upper third of lateral vaginal wall is spread directly on a clean microscope slide. The smear is immediately fixed with a cytological spray fixative or in an alcohol-ether dip. Fixation or preservation is one of the most important steps in the procedure.



Drying of the cells prior to fixation will usually result in artifacts such as nuclear distortion and vacuolization. After fixation there are no special handling requirements for cytological smears. However, smears should remain in the fixative for about one hour. A second clean glass slide may be placed on each fixed slide for protection.

Procedure:

Filter the Harris Haematoxylin immediately before use.

1. Dip slide(s) gently 5-10 times in 95% ethanol.

2. Dip slide(s) gently 5-10 times in 70% ethanol.

3. Dip slide(s) gently 5-10 times in distilled water.

4. Stain 5 minutes in Harris Hematoxylin.

5. Place smears in distilled water. Rinse in successive changes of distilled water

until the water remains colorless.

6. Dip slide(s) gently 5-10 times in 70% ethanol.

7. Dip slide(s) in a 1% solution of HCl in 70% ethanol until the smear shows a

Salmon color.

8. Rinse slide(s) well in 2 changes of 70% ethanol.

9. Dip slide(s) gently in a 3% solution of ammonium hydroxide in 70% ethanol

until the smear takes on a blue color.

10. Rinse the slide(s) in two changes of 70% ethanol.

11. Dip slide(s) 5-10 times in 95% ethanol.

12. Stain slide(s) in OG-6 for 2 minutes.

13. Rinse slide(s) in two changes of 95% ethanol.

14. Stain slide(s) in EA-50 or EA-65 for 3-6 minutes.

15. Rinse slide(s) well in two changes of 100% methanol.

16. Rinse slide(s) in one part absolute methanol one part xylene.

17. Clean smear in xylene mounting.

Procedure:

1. After the smear has been completely cleaned in xylene it is mounted with a

Microscope slide cover glass preferably 22x40mm, #1 thinness.

2. A permanent clean mounting medium should be used.

3. The excess xylene should be drained, in order to avoid the appearance of air

spaces when xylene evaporates.

4. Place the required amount of mounting medium along an edge of one of the

longer borders of the cover slip.

5. Place the slide at right angles to the edge of the cover slip so that the side containing the cells is facing the mounting medium.

6. Slowly lower the slide and permit the mounting medium to spread between the

slide and cover slip.

Results:

Nuclei are stained blue while cytoplasm displays varying shades of pink, orange, yellow and green.

Limitations:

1. Proper specimen collection and fixation of cells is essential for interpretation.

TRANS VAGINAL ULTRASOUND:

Before proceeding to transvaginal ultrasound, the woman was asked to empty the bladder. With patient in lithotomy position, 5 MHz vaginal probe was introduced into the vagina and the length & width of the cervix

was measured with the probe placed in the anterior fornix of the vagina. The appropriate sagital view of cervix was obtained by simultaneous imaging of external and internal os. External os was identified by its triangular echo density and internal os by its V-shape appearance. The cervical canal was seen as a translucent line connecting these two points. The distance between the external and internal os was taken as cervical length. The width was measured at the level of internal os. All these measurements were repeated thrice and the averaged readings were taken for statistical analysis. These measurements were repeated every four weeks till delivery.

To reduce the interobserver variability and improve reproducibility of cervical measurements using transvaginal ultrasound, the following criteria were adopted;

* The internal os is visualized as a flat dimple or an isosceles triangle.

* The whole length of the cervix is visualized.

* The whole length of the cervix is visualized.

* The external os appears symmetric

Method of Statistical Analysis:

The following methods of statistical analysis have been used in this study.The data were entered into a Microsoft Excel Worksheet and analysed using SPSS(ver 7.5) statistical package.

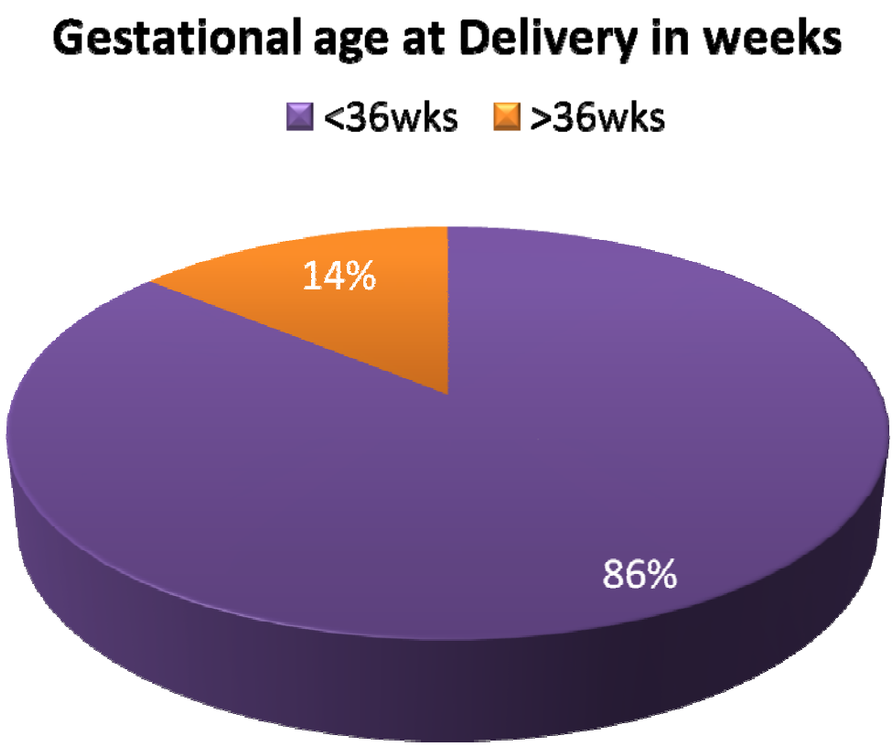
The results were presented in number and percentage in tables and figures.

Cervical length and Karyopyknotic Index are plotted in Chi Square Table and the results were analysed.

RESULTS AND ANALYSIS

RESULTS AND ANALYSIS:

TOTAL NO OF PATIENTS	GESTATIONAL AGE IN WEEKS	
	<36 WEEKS	>36 WEEKS
100	86	14

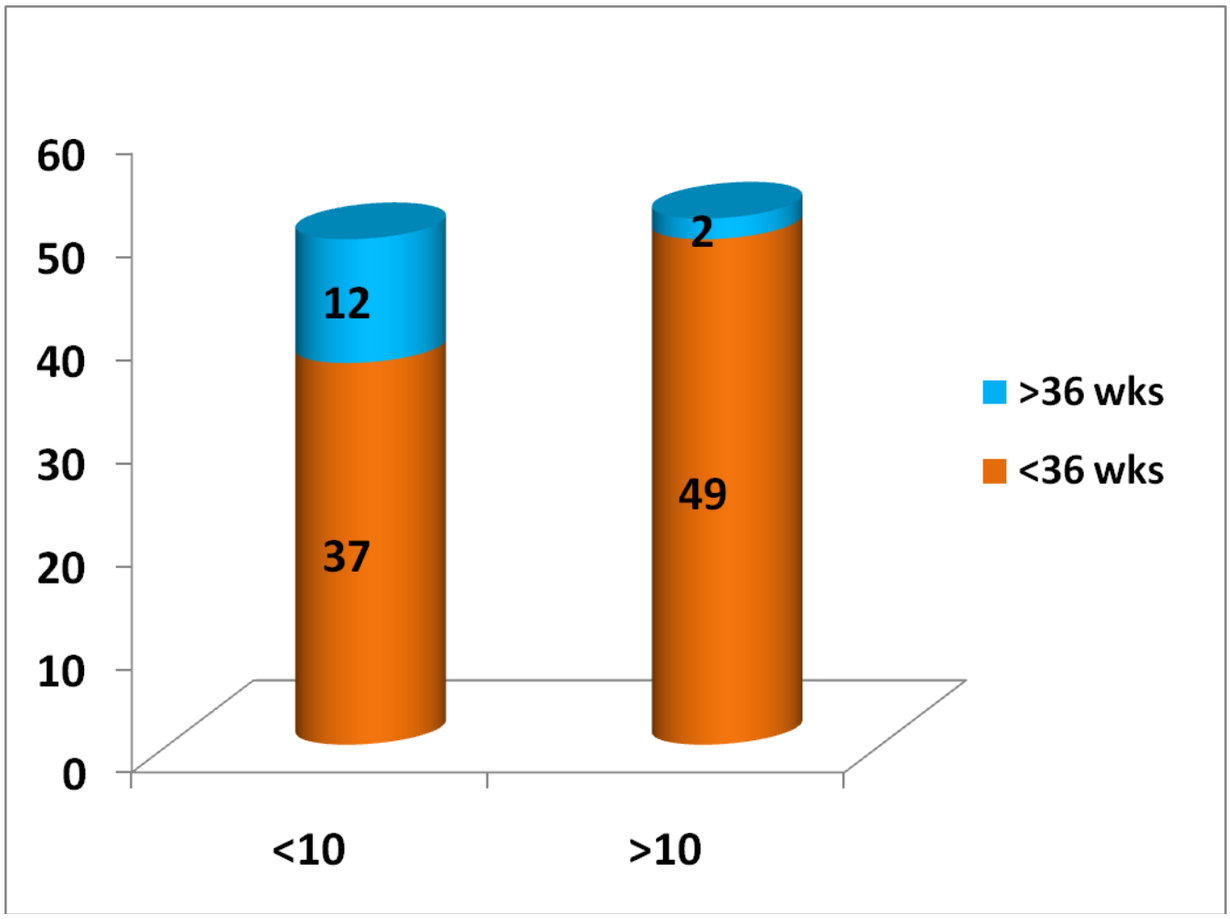


Among the 100 patients who had signs and symptoms of threatened preterm labour 86 (86%) delivered before 36 weeks and 14 (14%) delivered at term

KPI - Gestational age at Delivery in weeks

			Gestational age at Delivery in weeks		Total
			Below 36	Above 36	
KPI	Below 10	Count	37	12	49
		% within KPI	75.5%	24.5%	100.0%
		% within Gestational age at Delivery in weeks	43.0%	85.7%	49.0%
	Above 10	Count	49	2	51
		% within KPI	96.1%	3.9%	100.0%
		% within Gestational age at Delivery in weeks	57.0%	14.3%	51.0%
Total		Count	86	14	100
		% within KPI	86.0%	14.0%	100.0%
		% within Gestational age at Delivery in weeks	100.0%	100.0%	100.0%

KPI - Gestational age at Delivery in weeks

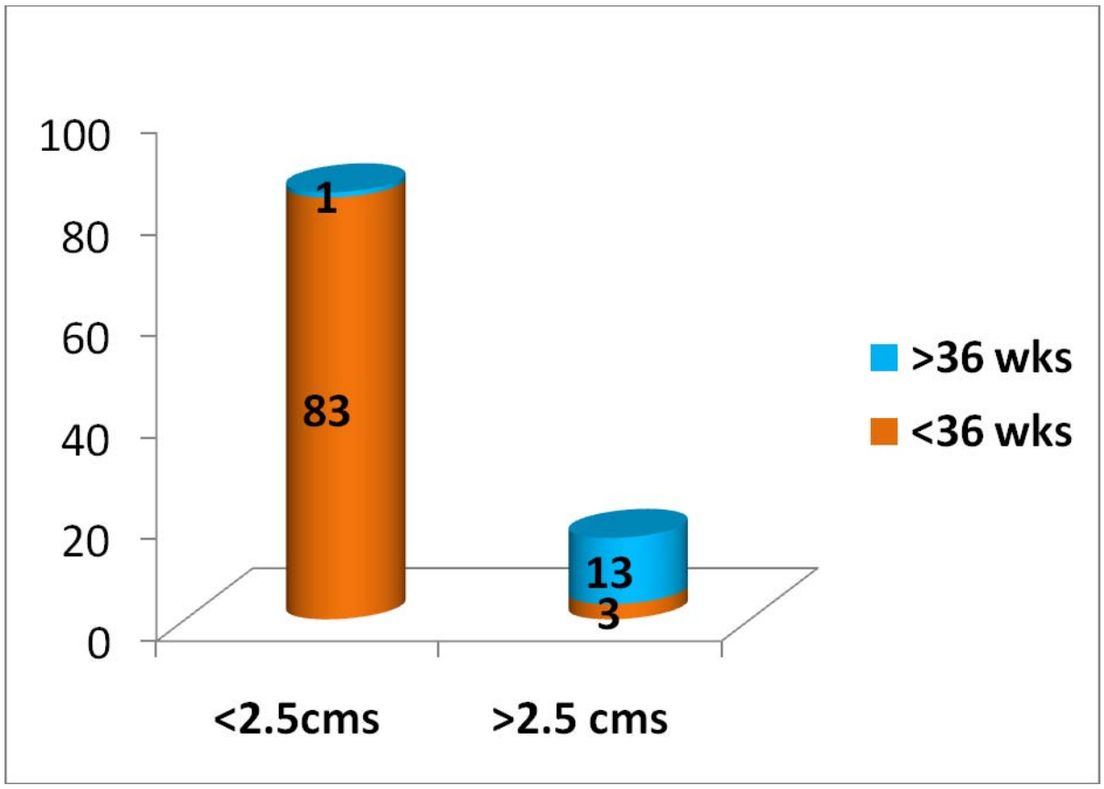


Among the vaginal cytology smears collected from 100 patients; 51 smears showed KPI >10 showing progesterone deficiency and impending delivery, of them 49(96.1%) delivered before 36weeks and 2(3.9%) delivered >36weeks. Remaining 49 smears showed KPI<10;of which 37(75.5%) delivered before 36 weeks and 12 (24.5%) delivered after 36 weeks. This shows significant **P value (0.003).**

Cervix Length in cms - Gestational age at Delivery in weeks

			Gestational age at Delivery in weeks		Total
			Below 36	Above 36	
Cervix Length in cms	Below 2.5	Count	83	1	84
		% within Cervix Length in cms	98.8%	1.2%	100.0%
		% within Gestational age at Delivery in weeks	96.5%	7.1%	84.0%
	Above 2.5	Count	3	13	16
		% within Cervix Length in cms	18.8%	81.3%	100.0%
		% within Gestational age at Delivery in weeks	3.5%	92.9%	16.0%
Total		Count	86	14	100
		% within Cervix Length in cms	86.0%	14.0%	100.0%
		% within Gestational age at Delivery in weeks	100.0%	100.0%	100.0%

Cervix Length in cms - Gestational age at Delivery in weeks

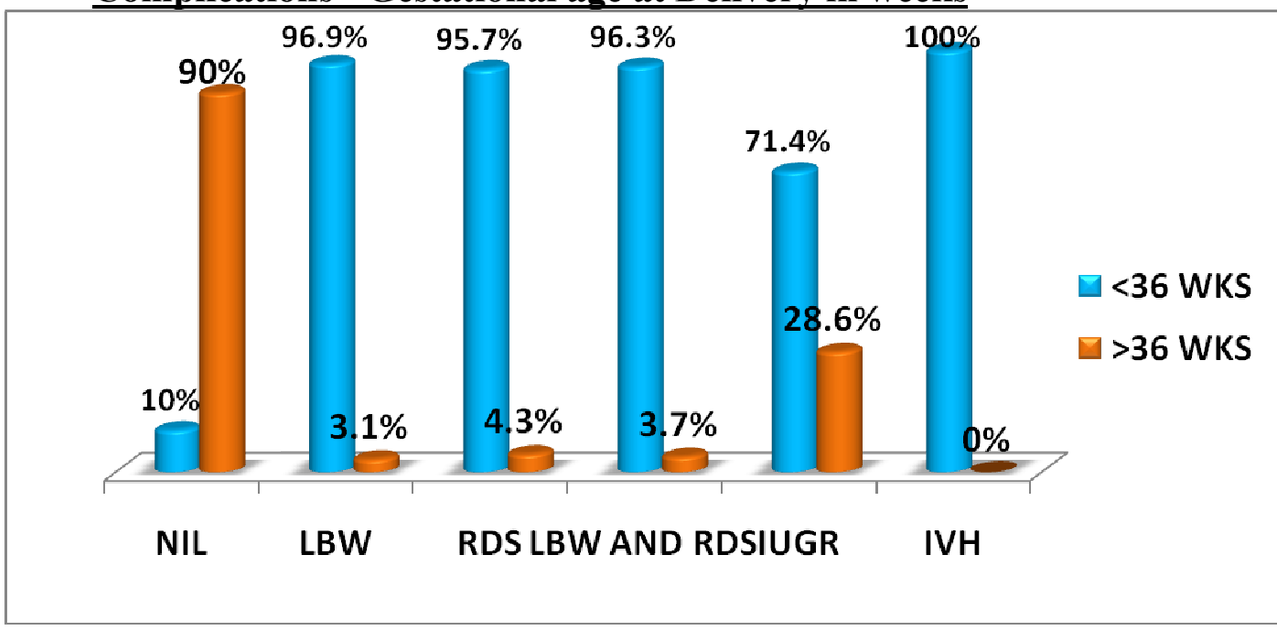


Among 100 patients; 86 patients delivered preterm of them 84 had cervical length <2.5cms; 83(98.8%) of them delivered before 36 weeks and 1(1.2%) delivered at term.; 16 had cervical length >2.5cms; 13(81.3%) delivered at term and 3(18.8%) delivered before 36 weeks. This shows highly significant **P value <0.001**.

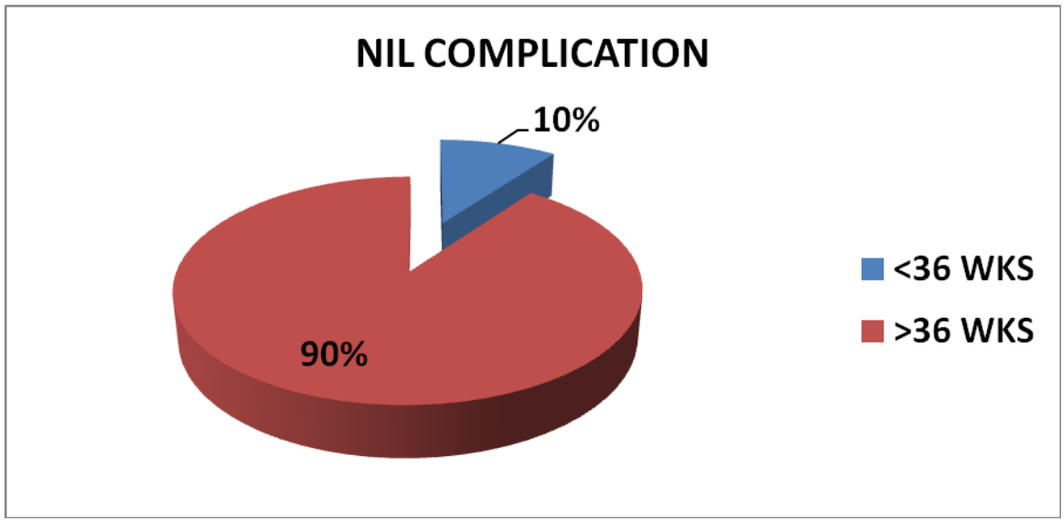
Complication * Gestational age at Delivery in weeks

			Gestational age at Delivery in weeks		Total
			Below 36	Above 36	
Complication	No complication	Count	1	9	10
		% within Complication	10%	90%	100.0%
		% within Gestational age at Delivery in weeks	1.2%	64.29%	11.0%
	LBW	Count	31	1	32
		% within Complication	96.9%	3.1%	100.0%
		% within Gestational age at Delivery in weeks	36.0%	7.14%	31.0%
	RDS	Count	22	1	23
		% within Complication	95.7%	4.3%	100.0%
		% within Gestational age at Delivery in weeks	25.6%	7.14%	23.0%
	LBW and RDS	Count	26	1	27
		% within Complication	96.3%	3.7%	100.0%
		% within Gestational age at Delivery in weeks	30.2%	7.14%	26.0%
	IUGR	Count	5	2	7
		% within Complication	71.4%	28.6%	100.0%
		% within Gestational age at Delivery in weeks	5.8%	14.29%	8.0%
	IVH	Count	1	0	1
		% within Complication	100.0%	.0%	100.0%
		% within Gestational age at Delivery in weeks	1.2%	.0%	1.0%
Total		Count	86	14	100
		% within Complication	86.0%	14.0%	100.0%
		% within Gestational age at Delivery in weeks	100.0%	100.0%	100.0%

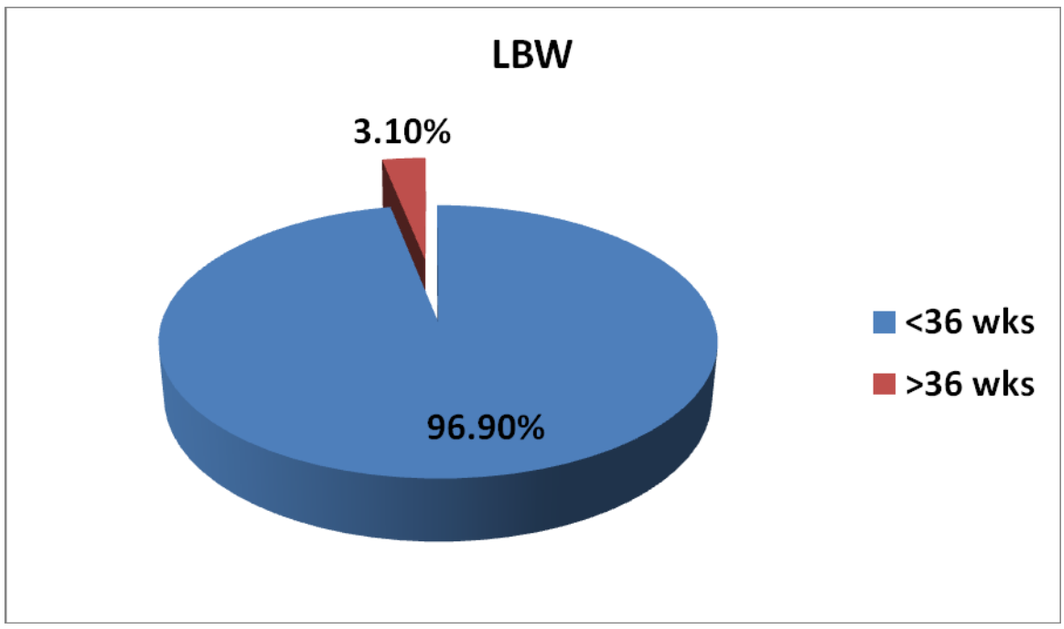
Complications - Gestational age at Delivery in weeks



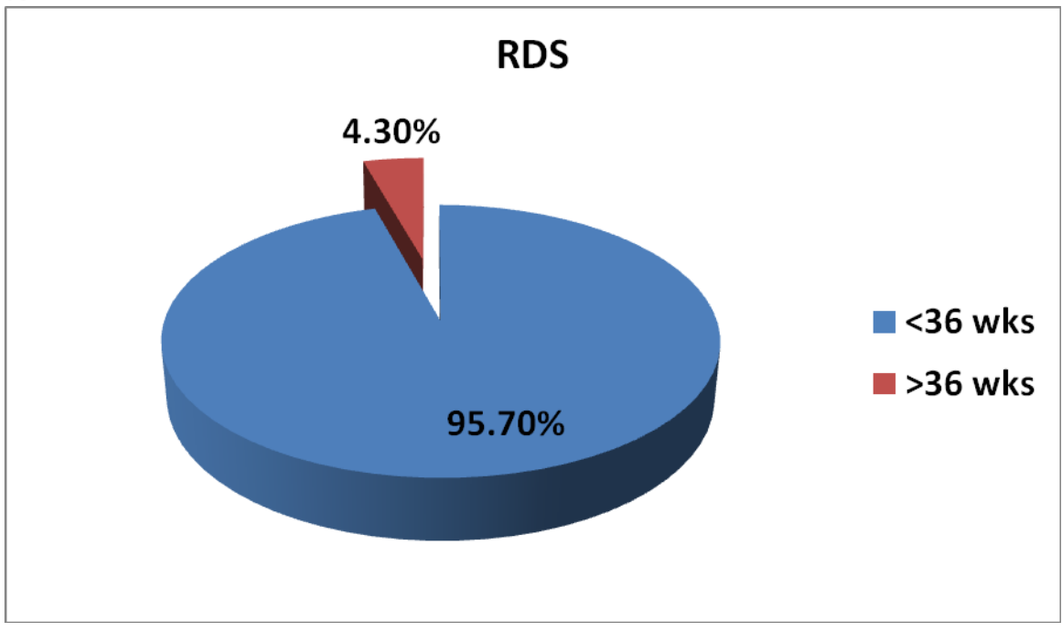
Among 100 patients delivered ; 86 babies delivered <36 weeks and 14 babies delivered at term; of them 90(90%) babies had different postnatal complications and 10(10%) babies had no complications.



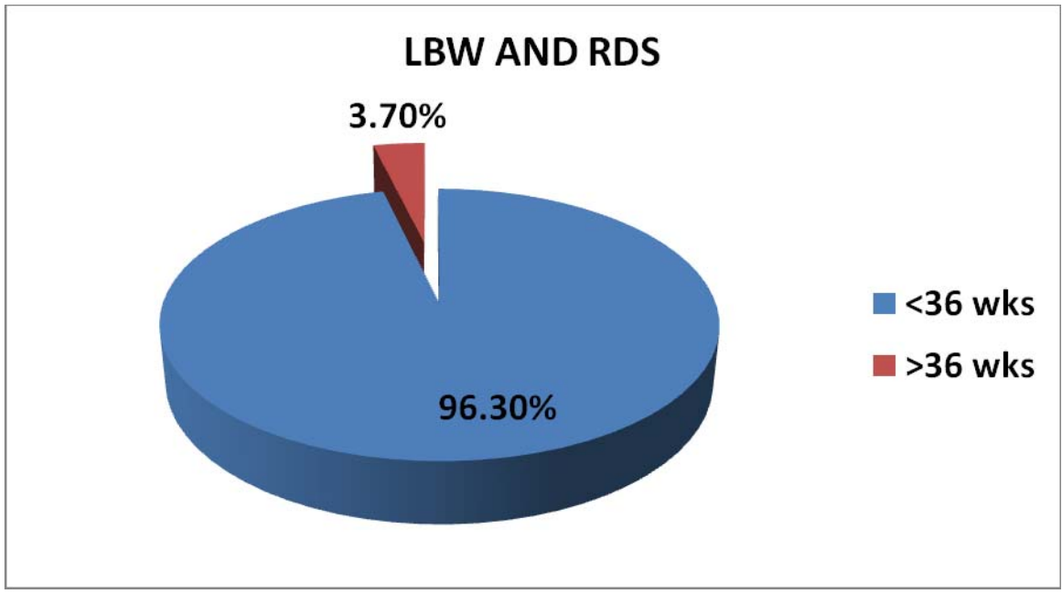
Among 100 patients delivered; 10 babies had no complication; of them 1(10%) delivered <36 wks and 9(90%) delivered at term



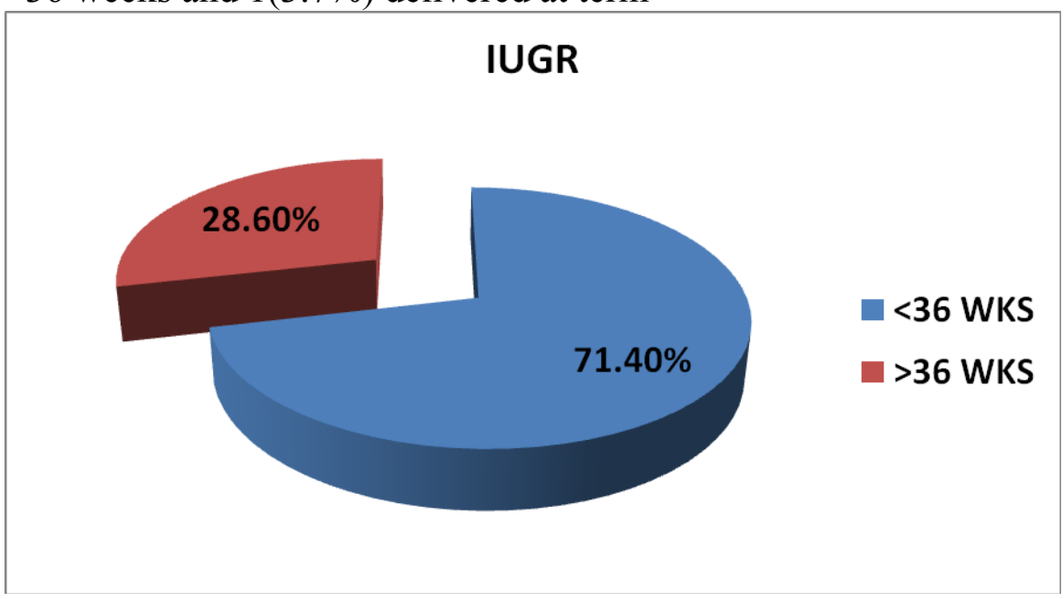
Among 100 patients delivered; 32 babies were Low Birth Weight; of them 31(96.9%) delivered <36 weeks and 1(3.1%) delivered at term



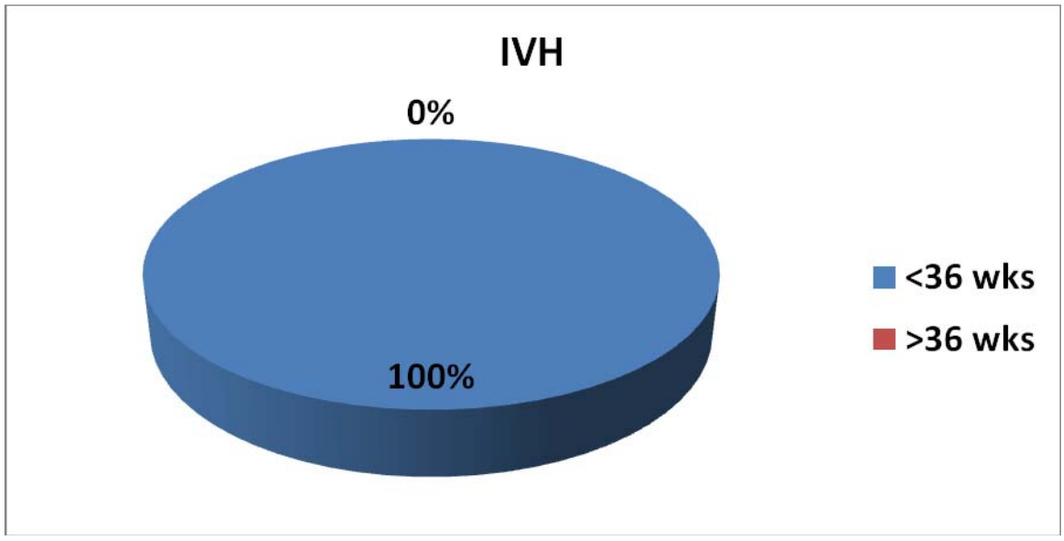
Among 100 patients delivered; 23 babies had Respiratory Distress Syndrome; of them 22(95.7%) delivered <36 weeks and 1(4.3%) delivered at term



Among 100 patients delivered; 27 babies had Low Birth Weight and Respiratory Distress Syndrome; of them 26(96.3%) delivered <36 weeks and 1(3.7%) delivered at term



Among 100 patients delivered; 7 babies had Intra Uterine Growth Retardation; of them 5(71.4%) delivered <36 weeks and 2(28.6%) delivered at term

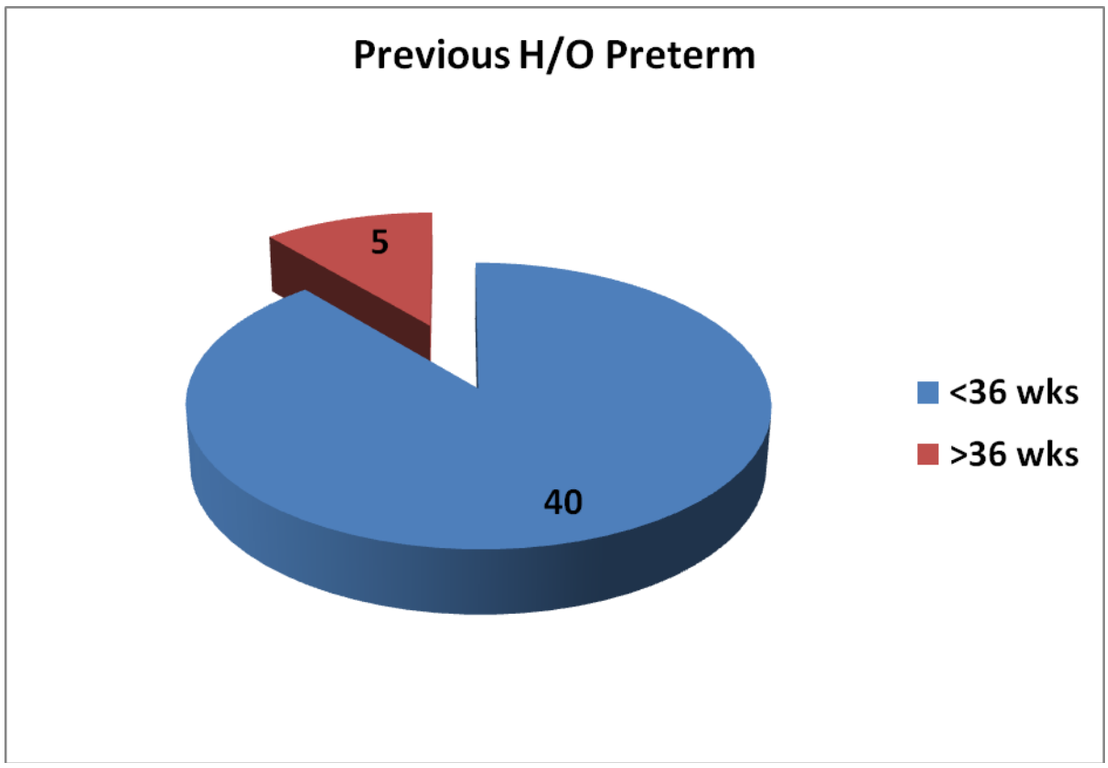


Among 100 patients delivered; 1 baby had Intraventricular hemorrhage which delivered at < 36 weeks.

Previous History of Preterm - Gestational age at Delivery in weeks

		Gestational Age at Delivery in weeks		Total
		Below 36	Above 36	
Positive	Count % within Previous H/O Preterm	40	5	45

Previous History of Preterm * Gestational age at Delivery in weeks

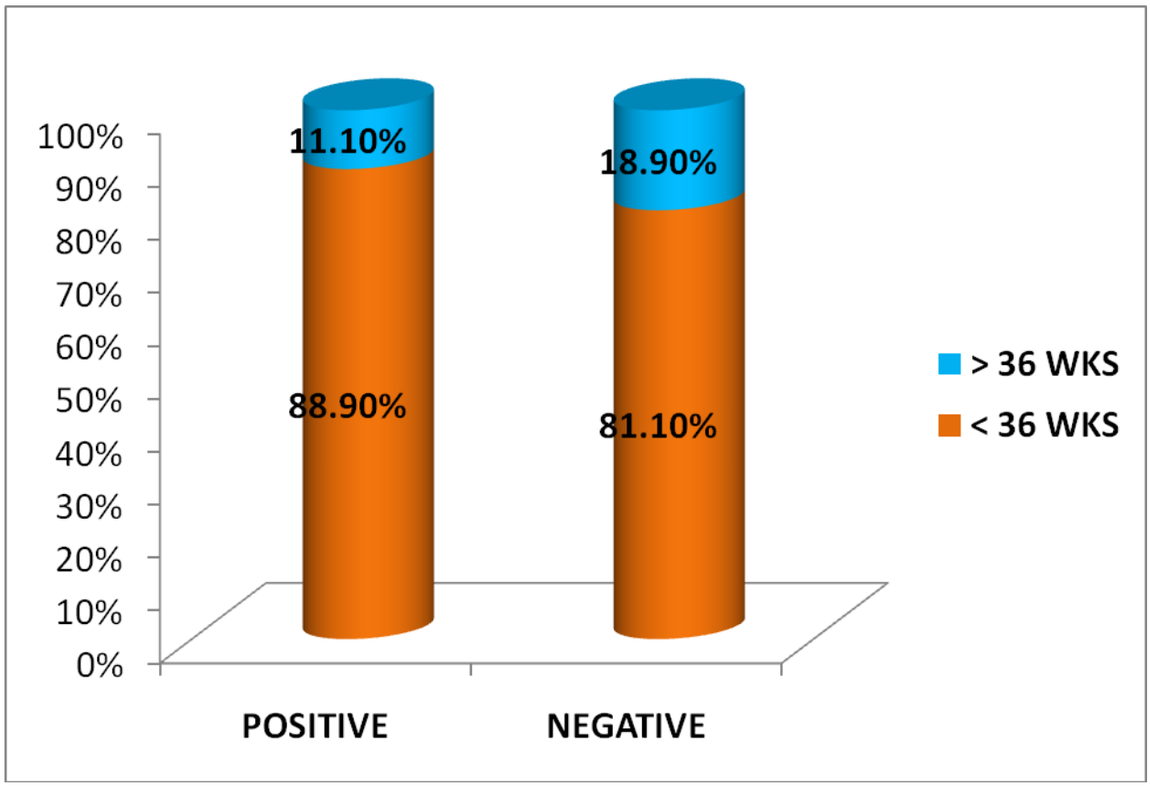


Among the 100 patients 45 of them had Previous H/O Preterm and 40 of them had Recurrent Preterm Labour and only 5 delivered at term. This shows **P value is highly significant i.e., <0.001**.

Genital Tract Infection * Gestational age at Delivery in weeks

			Gestational age at Delivery in weeks		Total
			Below 36	Above 36	
Genital Tract Infection	Positive	Count	56	7	63
		% within Genital Tract Infection	88.9%	11.1%	100.0%
		% within Gestational age at Delivery in weeks	65.1%	50.0%	63.0%
	Negative	Count	30	7	37
		% within Genital Tract Infection	81.1%	18.9%	100.0%
		% within Gestational age at Delivery in weeks	34.9%	50.0%	37.0%
Total		Count	86	14	100
		% within Genital Tract Infection	86.0%	14.0%	100.0%
		% within Gestational age at Delivery in weeks	100.0%	100.0%	100.0%

Genital Tract Infection - Gestational age at Delivery in weeks

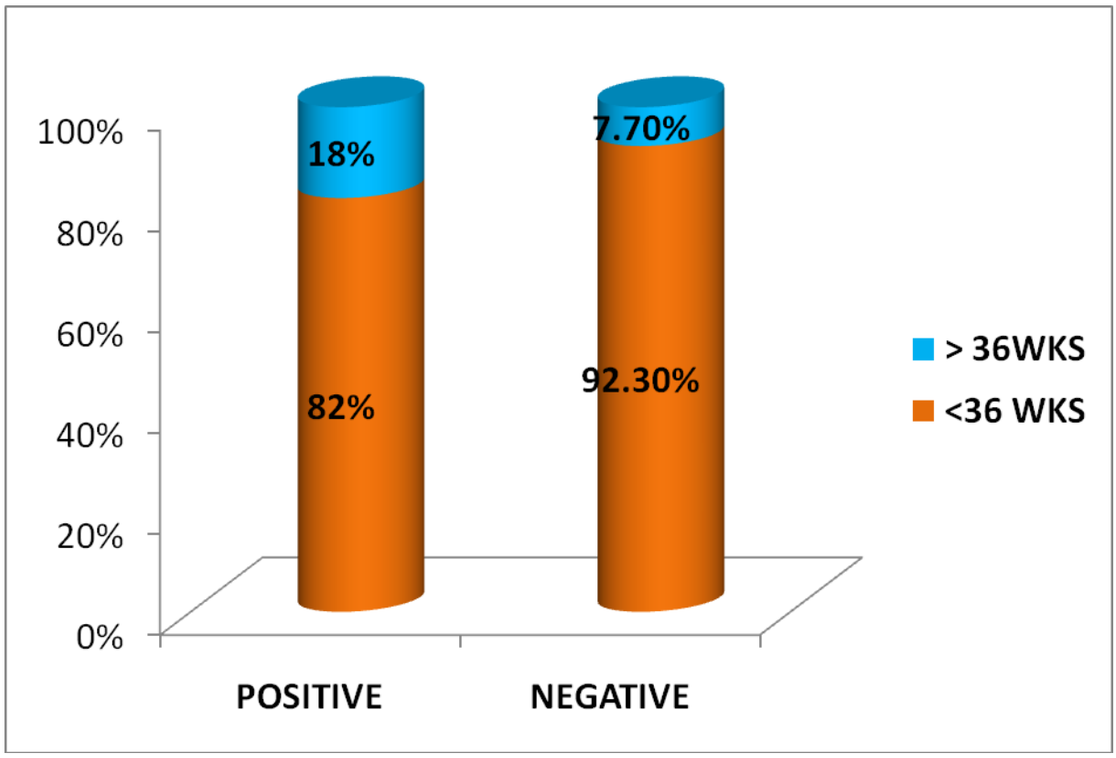


Among patients 100 patients, 63 patients had Genital Tract Infection; of them 56(88.9%) delivered before 36 weeks and 7(11.1%) delivered at term; 37 patients did not had Genital Tract Infection; of them 30 (81.1%) delivered before 36 weeks and 7(18.9%) delivered at term. So P value is insignificant.

Urine C/S - Gestational age at Delivery in weeks

			Gestational age at Delivery in weeks		Total
			Below 36	Above 36	
Urine C/S	Positive	Count	50	11	61
		% within Urine C/S	82.0%	18.0%	100.0%
		% within Gestational age at Delivery in weeks	58.1%	78.6%	61.0%
	Negative	Count	36	3	39
		% within Urine C/S	92.3%	7.7%	100.0%
		% within Gestational age at Delivery in weeks	41.9%	21.4%	39.0%
Total		Count	86	14	100
		% within Urine C/S	86.0%	14.0%	100.0%
		% within Gestational age at Delivery in weeks	100.0%	100.0%	100.0%

Urine C/S - Gestational age at Delivery in weeks

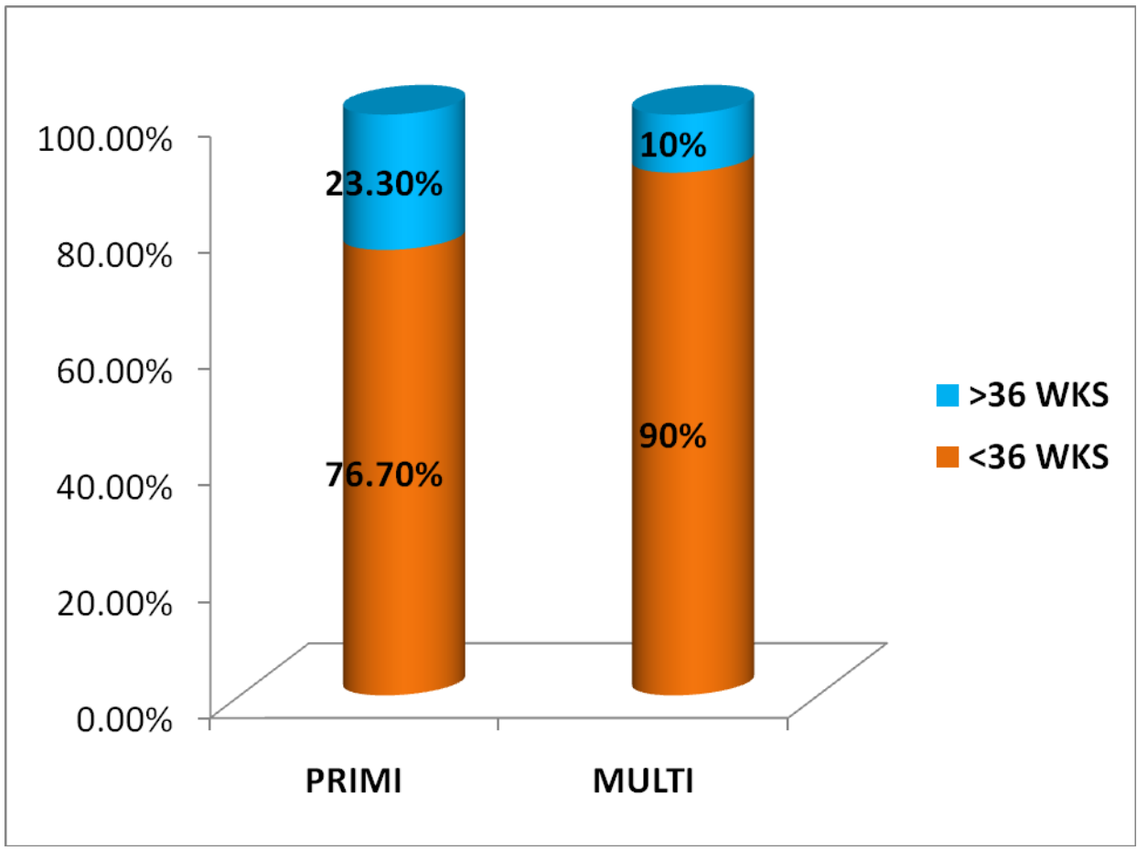


Among 100 patients examined; 61 patients has Positive Urine C/S; of them 50(82%) delivered before 36 weeks and 11(18%) delivered at term; 39 patients has Negative Urine C/S; of them 36(92.3%) delivered before 36 weeks and 3(7.7%) delivered at term. So P value is insignificant.

PARITY - Gestational age at Delivery in weeks

			Gestational age at Delivery in weeks		Total
			Below 36	Above 36	
PARITY	PRIMI	Count	23	7	30
		% within PARITY	76.7%	23.3%	100.0%
		% within Gestational age at Delivery in weeks	26.7%	50.0%	1.0%
	MULTI	Count	63	7	70
		% within PARITY	90.0%	.10%	100.0%
		% within Gestational age at Delivery in weeks	73.3%	50.0%	3.0%
Total		Count	86	14	100
		% within PARITY	86.0%	14.0%	100.0%
		% within Gestational age at Delivery in weeks	100.0%	100.0%	100.0%

PARITY - Gestational age at Delivery in weeks



Among 100 patients examined; 30 patients were PRIMI of them 23(76.7%) delivered before 36 weeks and 7(23.3%) delivered at term; 70 patients were MULTI of them 63(90%) delivered before 36 weeks and 7(10%) delivered at term. So P value is insignificant.

DISCUSSION

DISCUSSION

The studies of cervical length and karyopyknotic index for assessing the risk for preterm labour is very limited. By combining those two parameters increases the specificity. Vaginal cytology is a simple and inexpensive parameter as compared to estimation of urinary total oestrogen, ultrasound studies and amniotic fluid analysis. In order to increase the predictability of vaginal cytology, in addition to subjective, classification of the smears into 'pre-term' and 'at-term' patterns, eosinophilic and karyopyknotic indices can be calculated.

	Iams et al (1990)	Taipale P et al (1998):	Carvalho et al (2003)	Heath VC et al (1998)	Present study
Total pts	2531	3694	529	27	100
Initial cervical length assement done on	24wks	24wks	14wks	30wks	34wks
Preterm labour & its week	4.3% (<35wks)	2.4% (<34wks)	3.2% (<34wks)	74% (<36wks)	83% (<36wks)
Cutoff value of cervical length	<3.5cm	2.5cm	4.2 - 3.8cm	<2.5cm	<2.5cm

In most studies the addition of cervical funneling does not improve the predictive accuracy of cervical length in preterm labor prediction . This may, in part, be due to the wide variations noted in funnel measurement. Rust et al 14 have found that as a categorical variable (present or absent), a funnel is a significant risk factor for preterm labor. However, the latter study had a small sample size and was retrospective in nature. Additional prospective studies will be required to determine if the presence of a funnel adds to the predictive accuracy of cervical length in predicting preterm delivery.

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Cutoff value of cervical length	<3.5cm	2.5cm	4.2 - 3.8cm	<2.5cm	<2.5cm

	Pundel et al	Present study
KP index <10	414	49
Outcome of preterm KPI <10	4.3%	70%
KPI >10	574	51
Occurrence of preterm	92	96.1%

The Karyopyknotic index of vaginal cytology for assessing the time of delivery is very low cost method and it can be done in all centers. But the studies are very less. So the supportive evidence for this test is very less. By adding this to cervical lengthening increases the specificity for assessing the gestational age of pregnancy.

Very limited studies are available to explain the correlation between vaginal cytology and preterm birth .Pundel et all study clearly tells , if the KP index more than 10 they may go for preterm labour(92%) which is very much comparable to present study where the occurrence of preterm in KP index more than 10 is (96%).

Pandit et al studied the eosinophillic and karyopyknotic index to assess the preterm delivery. And the results are well correlating with our study to assess the preterm pregnancies

A similar study was done J.J.Group of Hospitals, Mumbai by collecting single smears from 75 pregnant women with features of preterm labour. In addition to this categorization of the smears, eosinophilic and karyopyknotic indices were calculated. For this investigation, a total of one hundred cells were counted in four different microscopic fields under high power lens (magnification 40 x 10).

The time interval between the day of obtaining the smear and the date of delivery was noted, and its relationship with the smear pattern was recorded. The data was analysed by discriminant analysis.

Ortner carried out discriminant analysis of eosinophilic and karyopyknotic indices and thus supplemented subjective criteria of cytological pattern determination with the objective criteria. The same method was followed by them. They found the values of eosinophilic and karyopyknotic indices were lower than those obtained by Ortner and T value was also low and could separate 'pre-term' and 'at-term' patterns quite well without any overlapping. Their prediction of onset of labour from the smear pattern was correct in 73% of the patients. Thus, they found that vaginal cytology at the end of pregnancy is a useful parameter to predict the onset of labour in most cases, supporting our study.

By doing both these investigations give clue to the risk of preterm pregnancy. By adding these investigation together increases the specificity

of assessing the risk of preterm pregnancy. Also we have shown previous H/O preterm birth increases the recurrence risk which was supported by Yost and co-workers as a patient's number of preterm births increases and the gestational age at preterm delivery decreases, the likelihood of another preterm delivery rises by 3 to 4-fold. They found that these historical variables in 22 patients did not affect the predictive accuracy of a cervical length < 25 mm between 16 and 19 weeks' gestation. However, because of the small sample size, the power of the study to detect a clinically significant difference was low. Until additional studies are reported, prior preterm delivery should be considered a categorical variable that increases the risk of preterm labor

Complications related to the preterm are very high. All are managed well by expecting the complications and be prepare for them.

Iams et all study explains the need of test to asses the gestational age by cervical length. Complication like IUD and LBW are very common. By doing this two techniques increases the sensitivity and specificity of the pt that whom are going for labour.

GENITAL TRACT INFECTION:

The young gynecology patient diagnosed with gonorrhea, chlamydia, or trichomoniasis has an approximate 25% risk of reinfection during the subsequent 12 months, but a clear association between these organisms and preterm delivery has not been established. BV is a vaginal syndrome associated with an alteration of the normal vaginal flora rather than an infection specific to any one organism and a lack of vaginal inflammation is evident when compared with vaginitis. The diagnosis of BV should be suspected with a positive Gram stain result or the presence of 3 of 4 traditional diagnostic signs (homogenous gray-white discharge, >20% clue cells on saline wet smear, positive whiff test, and a vaginal pH >4.50) Patients should be treated per the US Centers for Disease Control and Prevention guidelines, with test-of-cure sampling and subsequent treatment if necessary.

PRETERM LABOR/BIRTH HISTORY:

A history of prior preterm deliveries places the patient in the high-risk category. Of the predictors of preterm birth, past obstetric history may be one of the strongest predictors of recurrent preterm birth. Given a baseline risk of 10-12%, the risk of recurrent preterm birth after 1, 2, and 3 consecutive preterm births may be increased to approximately 15%, 30%,

and 45%, respectively. Pre-conceptual counseling should help encourage patients to make informed decisions concerning future pregnancy in light of prematurity risk in the presence of previous preterm delivery. Often the best time to counsel the patient is at her 4- to 6-week postpartum check after a preterm delivery.

In our study, complications like LBW, RDS, IUGR and IUD are common in preterm babies. Usually those preterm babies will go for high mortality and morbidity. In our study it's very less. Because, we were expected those preterm delivery and neonatal complications. Because of these we could able to reduce the morbidity and mortality of neonates of preterm.

Those patients who are admitted in hospital for threatened labour with KPI more than 10 and cervical length <2.5cm are very high risk patients for preterm labour so, those patients must be admitted in hospital and they should be started on tocolytic and other measures. The neonatal complications must be expected and the precautions have to be taken to prevent the mortality and morbidity of neonates.

Our study did not show any relation between parity and cervical dimensions. Gramellini et al (2002) reported similar finding in their cross sectional study involving 321 subjects. They showed that cervical length was comparable in nulliparous and parous women throughout pregnancy and

shows a progressive, linear reduction between the 10th and 40th weeks in both the groups. But some studies have reported that cervices of parous women are thicker and lengthier. They have attributed it to the fact that elastin, collagen and water content of parous cervices is different than nulliparous cervices because of changes that the cervix undergoes after delivery.

CONCLUSION

CONCLUSION:

The incidence of preterm birth remains around 12%. Preterm labor is a heterogeneous process. The presence of multiple interactive continuous variables explains the relatively low predictive value of any one variable. A transvaginal assessment of cervical length is one of the best tests for predicting preterm birth. A cervical length < 25 mm (10th percentile) between 28 to 36 weeks' gestation is generally agreed upon as increasing the risk of preterm labor. The shorter the cervical length, the greater the likelihood of preterm labor. The positive predictive value of a 25 mm cervix for preterm labor is dependent upon the patient's pretest probability (i.e. high-risk or low-risk for preterm labor).

Prior spontaneous preterm birth, fetal fibronectin, cervical length, and vaginal cytology are associated with early preterm birth. The association of the first 4 with preterm delivery is more than additive. These associations indicate that there is more than one pathway resulting in preterm delivery. In the future, a multifactorial assessment of patients at risk for preterm delivery should improve the positive predictive value of our testing schema. Specific therapeutic regimens depending upon the etiology or etiologies of preterm labor will be required and should improve our current limited successfully inhibition of preterm labor.

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ANNEXURES

ANNEXURES

PROFORMA

1.Patient particular:

Name: Hospital No:

Age: G P L A

D.O.A: I.P. No

2.Complaints:

H/O any imminent symptoms

3.Menstrual History:

Previous cycles

LMP

EDD

4.Marital History:

Married life

5.Obstetric History:

H/O any significant obstetric complications in the previous pregnancies

6.Past History of any medical or surgical illness

7.Family History:

Any History of Preterm in the mother, siblings or cousins

8.General Physical Examination:

Ht, Wt, Pallor, Edema, Breasts, Thyroid, PR, BP

9.Systemic Infections:

CVS

RS

P/A- Uterine size, liquor, FHR

10.Investigations:

Hb, Urine routine, (Plt count, LFT, RFT, Fundoscopy-if patient had high BP)

11. Cervical Length Measurement:

12.Vaginal Cytology Test Result:

13.GA at delivery

14.NN Outcome